Microbiota-Associated Metabolites in Tumor Immunity: Shared Regulatory Modules, Context-Dependent Outcomes, and Therapeutic Translation

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Scope and evidence classification of the supplementary materials.

The supplementary tables include metabolites, host-derived mediators, immune-cell interactions, cancer contexts, and translational strategies discussed in the main text. Molecules are classified according to biosynthetic origin as: Category I, metabolites directly synthesized by microorganisms; Category II, host metabolites modified by microbial enzymes; Category III, microbiota-dependent host co-metabolites requiring sequential microbial and host metabolism; and Category IV, predominantly host- or tumor-derived mediators whose abundance or signaling may be indirectly influenced by microbiota-associated metabolic networks. Evidence levels follow the hierarchy used in the main text: Level I, direct human administration of a chemically defined metabolite; Level II, mechanistic in vivo evidence with defined metabolite exposure and pathway- or cell-specific perturbation; Level III, ex vivo or in vitro mechanistic evidence; and Level IV, human observational evidence without direct metabolite intervention. Microbiota-directed interventions are considered indirect evidence for a specific metabolite unless altered metabolite exposure and causal mediation are demonstrated. Non-cancer models are included only as mechanistic precedents and are explicitly distinguished from cancer-specific evidence.

General notation and conventions. ↑ indicates an increase in the specified abundance, activity, expression, differentiation, or function; ↓ indicates a decrease; ↑↓ indicates context-dependent enhancement or suppression; and → indicates a mechanistic sequence, downstream effect, or phenotypic transition. Concentrations refer to the specific biological compartment or experimental system indicated and should not be compared directly across luminal or fecal, portal, peripheral-blood, tissue, intracellular, and in vitro settings. μM denotes micromolar and mM denotes millimolar. Evidence Levels I–IV follow the definitions provided above.

Table S1: Classification, biosynthetic origin, and immunomodulatory features of microbiota-associated metabolites and related host-derived mediators

Metabolite class	Representative metabolites	Representative microbial and/or host sources	Primary site of production or metabolism	Key biosynthetic pathway and biosynthetic origin	Representative immune effects	Concentration and exposure context	References
SCFAs	1. Core SCFAs: acetate (C_2, ~60%), propionate (C_3, ~20%), and butyrate (C_4, ~20%) 2. Minor SCFAs and branched-chain fatty acids include isobutyrate, isovalerate, and 2-methylbutyrate	1. Representative butyrate-producing bacteria: Family Ruminococcaceae: *Faecalibacterium prausnitzii*, Butyricicoccus pullicaecorum Family Lachnospiraceae: Eubacterium rectale, Genus Roseburia (R. inulinivorans, R. intestinalis), Anaerostipes hadrus, Coprococcus catus (via acrylate pathway),	1. Primary site: Colon, particularly the proximal colon, where microbial biomass and carbohydrate fermentation are highest 2. Secondary site: Small intestine, where microbial SCFA production is substantially lower because of the lower microbial biomass	Biosynthetic origin: Category I, metabolites directly synthesized by microorganisms. I. Carbohydrate Fermentation Pathway (Major Pathway) 1. Butyrate Biosynthesis Core Precursors: Glucose, fructose, inulin, pectin, and other non-digestible carbohydrates Key Steps: Glycolysis → pyruvate → acetyl-CoA → condensation of	1. Promotes Treg differentiation and IL-10 production through HDAC inhibition and GPR43/FFAR2-associated signaling. 2. Modulates macrophage and DC inflammatory programs and antigen-presenting functions. 3. Supports intestinal barrier-associated immune homeostasis. 4. Regulates B-cell differentiation and	1. Physiological Concentration Range Total SCFAs in colonic lumen: 50-200 mM (acetate ~60%, propionate ~20%, butyrate ~20%) Portal vein concentration: Butyrate 5-10 μM, propionate 32 μM, acetate 98-143 μM (liver efficiently uptakes butyrate/propionate; acetate distributes systemically) Venous blood concentration: Butyrate 0.5-3.3 μM, propionate 3.8-5.4 μM, acetate 100-150 μM 2.	[1-4]

	Coprococcus eutactus (via ButK pathway) Family Erysipelotrichaceae: Holdemanella biformis 2. Representative propionate-producing bacteria: Phylum Bacteroidetes: Bacteroides uniformis, B. vulgatus, Prevotella copri (via succinate pathway), Alistipes putredinis Phylum Firmicutes (Class Negativicutes): Phascolarctobacterium succinatutens (succinate →	acetyl-CoA to acetoacetyl-CoA → stepwise reduction to butyryl-CoA Terminal Steps: CoAT Pathway (most dominant bacteria): Butyryl-CoA:acetate CoA-transferase (requires acetate as a cosubstrate, e.g., F. prausnitzii, Roseburia spp.) ButK Pathway (few bacteria): Phosphotransbutyrylase^{+} butyrate kinase (e.g., Coprococcus eutactus, Subdoligranulum variabile) 2. Propionate Biosynthesis Succinate Pathway (Bacteroidetes, Negativicutes):	IgA production.	Concentration-dependent and context-dependent effects Butyrate: Low concentration (50-800 μM): Serves as the main energy source for colonic epithelial cells (IECs, accounting for 73-75% of IEC oxygen consumption), promoting stem cell proliferation Medium concentration (1-5 mM): Exerts HDAC inhibitory effects, regulating gene expression (e.g., TGF-β, Foxp3) High concentration (>5 mM): Inhibits cancer cell proliferation (via Warburg effect, inducing ROS and cell cycle arrest), with stronger HDAC inhibition

	propionate), Veillonella spp. (lactate → propionate), Megasphaera elsdenii (via acrylate pathway) Family Lachnospiraceae: Coprococcus catus (via acrylate pathway), Roseburia inulinivorans, Blautia obeum (via 1,2-propanediol pathway) Phylum Verrucomicrobia: *Akkermansia muciniphila* (via 1,2-propanediol pathway) 3. Representative acetate-producing	Carbohydrates → pyruvate → oxaloacetate → succinate → succinyl-CoA → propionate (requires vitamin B_{12}) Acrylate Pathway (Megasphaera elsdenii, Coprococcus catus): Lactate → lactyl-CoA → acrylyl-CoA → propionyl-CoA → propionate 1,2-Propanediol Pathway (Roseburia inulinivorans, A. muciniphila): Deoxysugars (fucose, rhamnose) → 1,2-propanediol → propionyl-CoA → propionate (requires vitamin B_{12}, occurs	(dependent on non-ACL pathways) Propionate: Low concentration (μM level): Regulates Treg via GPR43; High concentration (mM level): Inhibits tumor cell proliferation (e.g., hepatocellular carcinoma cells) Environmentally Regulated Concentration: Acidic pH (~5.5): Promotes butyrate production (inhibits Bacteroidetes, favors Firmicutes) Neutral pH (~6.7): Promotes propionate production (Bacteroidetes dominance) Iron deficiency: Reduces butyrate-producing bacteria such as

	bacteria: Most intestinal anaerobes: Genus *Bifidobacterium* (B. adolescentis, B. longum), most Firmicutes (e.g., Clostridium spp.), Bacteroidetes spp	in polyhedral bodies to sequester the toxic intermediate propanal) 3. Acetate Biosynthesis - Glycolysis → pyruvate → acetyl-CoA → acetyl phosphate → acetate (ATP production via substrate-level phosphorylation); or acetyl reduction (a universal pathway in most bacteria) II. Amino Acid Fermentation Pathway (Minor Pathway) Butyrate: Glutamate (via 3-methylaspartate pathway / 4-aminobutyrate pathway), lysine	Roseburia spp., decreasing butyrate concentration; sufficient iron promotes butyrate production

				(Intestinimonas AF211), histidine, cysteine Propionate: Aspartate (via oxaloacetate / fumarate), alanine (→ pyruvate → lactate → acrylate pathway), threonine/methionine (→ 2-oxobutyrate → propionyl-CoA)			
Bile Acids	1. Primary Bile Acids: Cholic acid (CA), CDCA, hyodeoxycholic acid (HCA), α-muricholic acid (αMCA, rodent-specific), β-muricholic acid (βMCA, rodent-specific) 2. Secondary Bile Acids: DCA, LCA, UDCA, isodeoxycholic acid (isoDCA), isoallolithocholic acid (isoalloLCA),	1. Host source: Hepatocytes synthesize primary BAs. 2. Representative microbial modifiers: BSH-producing Bifidobacterium, Lactobacillus, Clostridium, Enterococcus, and Bacteroides spp.;	1. Primary BA synthesis: Liver, predominantly hepatocytes 2. Microbial BA transformation: Terminal ileum and colon 3. Enterohepatic processing: Intestinal lumen, intestinal epithelium, portal circulation, and liver	1. Biosynthetic origin: Primary BAs are host-derived precursors, whereas secondary and iso-bile acids are Category II metabolites generated through microbial modification of host-derived BAs 2. Primary Bile Acid	1. Representative receptor- and cell-specific immune effects FXR and TGR5 signaling modulate inflammasome, NF-κB, macrophage, and cytokine programs. 3-oxoLCA inhibits	1. Physiological exposure context Metabolic Regulation: UDCA protects hepatocytes (anti-apoptosis); DCA activates TGR5 to promote GLP-1 secretion (improving insulin resistance) Immune Protection: 3-oxoLCA/isoalloLCA maintains Th17/Treg	[5, 6]

3-oxolithocholic acid (3-oxoLCA) 3. Special Derivatives: Microbially conjugated bile acids (MCBAs), sulfated bile acids (3-sulfo-LCA), allo-bile acids (alloCDCA, alloDCA)	bai-operon-containing bacteria such as Clostridium scindens, Clostridium hylemonae, and Peptacetobacter hiranonis; and HSDH-producing bacteria including Eggerthella lenta, Blautia producta, and Ruminococcus gnavus 3. 7α/β-Dehydroxylase (bai operon)-producing bacteria: *Clostridium scindens*, *Clostridium hylemonae*, and *Peptacetobacter hiranonis*	Synthesis Classic Pathway (90% in humans): Cholesterol undergoes 7α-hydroxylation by CYP7A1 (rate-limiting enzyme) → sterol ring modification → side-chain cleavage → conjugation with glycine/taurine (via BAT enzyme), generating CA-CoA and CDCA-CoA Alternative Pathway (10% in humans): Cholesterol undergoes C27 oxidation by CYP27A1 → 7α-hydroxylation by CYP7B1, generating only CDCA 3. Secondary Bile	Th17-cell differentiation through RORγt, whereas isoalloLCA and isoDCA can favor Treg-associated immune programs. LCA and related BA derivatives can engage VDR, FXR, PXR, and other nuclear-receptor pathways in a context-dependent manner 2. Immune Regulation: LCA activates VDR, inducing CYP3A4/SULT2A1 (bile acid detoxification); MCBAs regulate	balance, inhibiting intestinal inflammation 2. Elevated or pathological exposure context Cytotoxicity: High LCA/DCA damages cell membranes (promoting ROS generation), induces hepatocyte apoptosis (cholestasis) Pro-Inflammatory and Carcinogenic: High DCA activates Epidermal Growth Factor Receptor (EGFR)/ERK pathway (promoting CRC); high LCA induces DNA adducts (increasing hepatocellular carcinoma, HCC risk) Metabolic Disorders: Excessive CA/CDCA inhibits insulin signaling (Akt dephosphorylation), exacerbating T2DM

formerly *Clostridium hiranonis*	Acid Synthesis	FXR/PXR activity, affecting intestinal mucosal immunity
4. Hydroxysteroid Dehydrogenase (HSDH)-Producing Bacteria: Eggerthella lenta, Blautia producta, Ruminococcus gnavus, Clostridium absonum	Deconjugation: BSH enzyme hydrolyzes glycine/taurine conjugation bonds, releasing free bile acids	
5. Other Metabolizing Bacteria: Odoribacteraceae (produces isoalloLCA), Enterocloster bolteae (produces MCBAs)	7α/β-Dehydroxylation: bai operon (baiA2/baiCD/baiE, etc.) catalyzes CA → DCA, CDCA → LCA	
	Epimerization: HSDHs catalyze oxidation-reduction of hydroxyl groups at positions 3/7/12, generating isoDCA (3β-OH), UDCA (7β-OH)	
	Special Modifications: MCBAs, sulfation (SULT2A1), C3/C12 dehydroxylation	

				(Genus Clostridium)			
Polyamines	1. Core Polyamines: Putrescine, spermidine, spermine 2. Key Metabolites: N^1-acetylspermidine, N^1,N^{12}-diacetylspermine, 3-aminopropanal (3-AP, degradation product)	1. Representative microbial sources: Escherichia coli, Enterococcus faecalis, Lactobacillus curvatus, Bifidobacterium animalis subsp. lactis, and other intestinal bacteria. Host and tumor cells can also contribute to polyamine metabolism through endogenous polyamine-synthesis pathways 2. Others: Enterotoxigenic *Bacteroides fragilis* (ETBF), *H. pylori* (induces	1. Microbial production: Primarily the large intestine (colon), where microbiota-derived polyamines are absorbed into the bloodstream via colonic epithelium 2. Local metabolism: Intestinal and gastric mucosal compartments; endogenous polyamine metabolism can additionally occur in host tissues (site of *H. pylori* colonization, affecting local polyamine metabolism)	1. Biosynthetic origin: Source-dependent. Polyamines can arise from microbial metabolism as well as endogenous host-associated biosynthetic pathways and should therefore not be uniformly classified as directly microbially synthesized metabolites 2. Puu Pathway in E. coli: Putrescine undergoes γ-glutamylation (catalyzed by YcjK) → γ-glutamylputrescine → γ-glutamyl-γ-aminob	1. Modulates Treg differentiation and T-cell activation 2. Regulates NK-cell metabolic fitness and cytotoxic function 3. Influences macrophage polarization and inflammatory cytokine production 4. Modulates DC cross-presentation and other antigen-presenting functions	Reported polyamine effects vary substantially with metabolite identity, concentration, cell type, and experimental system. Micromolar concentrations have been used in selected immune and autophagy studies, whereas millimolar or higher experimental exposures can produce distinct proliferative, oxidative-stress, or immunoregulatory effects. These ranges should not be interpreted as universal physiological or tumor-specific thresholds	[7-10]

polyamine-degrading enzymes)	utyric acid (GABA) → GABA (requires OrdL, AldH, YcjL enzymes) 3. Arginine-Agmatine Pathway: A combined system of E. coli (AdiA converts arginine to agmatine, AdiC transports it) + Enterococcus faecalis (AguA converts agmatine to putrescine, AguD transports it) 4. Classic Synthesis Pathway: Ornithine → putrescine (catalyzed by ODC) → spermidine (catalyzed by SRM) → spermine (catalyzed by SMS), requiring dcAdoMet

				as the aminopropyl donor			
B Vitamins	1. Vitamin B1 (Thiamine pyrophosphate, free thiamine) 2. Vitamin B2 (Flavin mononucleotide, flavin adenine dinucleotide, free riboflavin) 3. Vitamin B3 (Niacin, nicotinamide, nicotinamide adenine dinucleotide) 4. Vitamin B5 (Coenzyme A, free pantothenic acid) 5. Vitamin B6 (Pyridoxal-5'-phosphate, pyridoxine, pyridoxamine) 6. Vitamin B7 (Biotin) 7. Vitamin B9 (Tetrahydrofolate, folic acid) 8. Vitamin B12 (Adenosylcobalamin, methylcobalamin,	Representative microbial sources vary across B-vitamin classes and include members of Bacteroides, Prevotella, Bifidobacterium, Lactobacillus, Clostridium, Escherichia, and related intestinal genera. Microbial biosynthetic capacity is vitamin- and strain-specific	Microbial B-vitamin synthesis occurs primarily within the intestinal microbiota, particularly in the colon. Dietary and microbiota-derived B vitamins have different sites and efficiencies of host absorption, and their systemic availability depends on vitamin identity and intestinal location	Biosynthetic origin: Microbiota-derived B vitamins represent Category I metabolites when directly synthesized by intestinal microorganisms, whereas dietary B vitamins are exogenous nutrients and should not be considered microbiota-derived metabolites. Biosynthetic pathways are vitamin-specific and involve distinct precursor and cofactor pathways for thiamine, riboflavin, niacin, pantothenate,	Supports immune-cell energy and one-carbon metabolism. Selected B vitamins regulate B-cell, Treg, $CD8^+$ T-cell, NK-cell, DC, and macrophage functions. Riboflavin-derived metabolites participate in MAIT-cell activation, whereas folate supports Treg maintenance. The relevance of individual microbiota-derived B vitamins to	B-vitamin effects depend on vitamin identity, nutritional status, microbiota-derived production, host absorption, and experimental dose. Deficiency, physiological sufficiency, and pharmacological supplementation represent biologically distinct exposure states. Reported dietary, circulating, tissue, and in vitro exposure levels should not be interpreted as directly equivalent, and tumor-specific concentration thresholds remain insufficiently defined.	[11-14]

	cyanocobalamin)			pyridoxal phosphate, biotin, folate, and cobalamin.	tumor immunity remains less well established than that of SCFAs, BAs, and Trp-derived metabolites.		
Microbiota-associated Trp metabolites and related host-derived mediators	Microbial Trp-derived metabolites, including IAA, ILA, IPA, I3A, and IDA; and the predominantly host- or tumor-derived Trp metabolite Kyn	Microbial Trp-derived metabolites: Specific producing taxa vary according to the metabolite and pathway. Kyn: Predominantly generated by host immune, stromal, or tumor cells through IDO1/TDO2-associated Trp metabolism; the microbiota can indirectly	Microbial Trp metabolism: Primarily the intestinal lumen and mucosa. Host Kyn metabolism: Immune, stromal, and tumor tissues, with systemic and local Trp–Kyn metabolism contributing to pathway activity.	Biosynthetic origin: Microbial indole derivatives are Category I metabolites directly synthesized by microorganisms from Trp. In contrast, Kyn is a Category IV, predominantly host- or tumor-derived mediator generated mainly through IDO1/TDO2-dependent Trp metabolism; microbiota-associated changes in Trp availability may indirectly modify	Microbial Trp-derived metabolites can modulate AhR-dependent immune-cell programs in a metabolite- and cell-specific manner, whereas host/tumor-derived Kyn and local Trp depletion can promote immunosuppressive signaling and restrict effector T-cell function. The final immune	Dose- and concentration-dependent effects vary across intestinal, circulating, tumor-tissue, and intracellular compartments and among experimental systems. Standardized tumor-specific concentration thresholds remain unavailable, and fecal or circulating abundance should not be interpreted as equivalent to effective target-cell exposure	[15]

	influence Trp availability and Kyn-pathway activity	this pathway	outcome depends on metabolite identity, concentration, responding cell type, and tumor context

Abbreviations: 3-AP, 3-aminopropanal; 3-oxoLCA, 3-oxolithocholic acid; 3-sulfo-LCA, 3-sulfolithocholic acid; ACL, ATP citrate lyase; AhR, aryl hydrocarbon receptor; AKT, AKT serine/threonine kinase; alloCDCA, allochenodeoxycholic acid; alloDCA, allodeoxycholic acid; ATP, adenosine triphosphate; BA, bile acid; BAs, bile acids; bai, bile acid-inducible operon; BAT, bile acid-CoA acid N-acyltransferase; BSH, bile salt hydrolase; ButK, butyrate kinase; C_2, acetate; C_3, propionate; C_4, butyrate; CA, cholic acid; CA-CoA, cholic acid-CoA thioester; CD, cluster of differentiation; CDCA, chenodeoxycholic acid; CDCA-CoA, chenodeoxycholic acid-CoA thioester; CoA, coenzyme A; CoAT, butyryl-CoA CoA-transferase; CRC, colorectal cancer; CYP7A1, cholesterol 7α-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; CYP27A1, sterol 27-hydroxylase; dcAdoMet, decarboxylated S-adenosylmethionine; DC, dendritic cell; DCA, deoxycholic acid; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; ETBF, enterotoxigenic Bacteroides fragilis; FFAR2, free fatty acid receptor 2; FOXP3, forkhead box P3; FXR, farnesoid X receptor; GABA, γ-aminobutyric acid; GLP-1, glucagon-like peptide 1; GPR43, G protein-coupled receptor 43; HCA, hyodeoxycholic acid; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; HSDH, hydroxysteroid dehydrogenase; I3A, indole-3-aldehyde; IAA, indole-3-acetic acid; IDA, trans-3-indoleacrylic acid; IDO1, indoleamine 2,3-dioxygenase 1; IEC, intestinal epithelial cell; IECs, intestinal epithelial cells; IgA, immunoglobulin A; IL-10, interleukin-10; ILA, indole-3-lactic acid; IPA, indole-3-propionic acid; isoalloLCA, isoallolithocholic acid; isoDCA, isodeoxycholic acid; Kyn, kynurenine; LCA, lithocholic acid; MAIT, mucosa-associated invariant T cell; MCBA, microbially conjugated bile acid; MCBAs, microbially conjugated bile acids; NF-κB, nuclear factor-κB; NK, natural killer; ODC, ornithine decarboxylase; Puu, putrescine-utilization pathway; PXR, pregnane X receptor; ROS, reactive oxygen species; RORγt, retinoic acid-related orphan receptor γt; SCFA, short-chain fatty acid; SCFAs, short-chain fatty acids; SMS, spermine synthase; SRM, spermidine synthase; SULT2A1, sulfotransferase family 2A member 1; T2DM, type 2 diabetes mellitus; TDO2, tryptophan 2,3-dioxygenase 2; TGF-β, transforming growth factor-β; TGR5, G protein-coupled bile acid receptor 1; Th17, T helper 17 cell; Treg, regulatory T cell; Trp, tryptophan; UDCA, ursodeoxycholic acid; VDR, vitamin D receptor; αMCA, α-muricholic acid; βMCA, β-muricholic acid.

Notes: Metabolites and related mediators are classified according to biosynthetic origin. Category I comprises metabolites directly synthesized by microorganisms; Category II comprises host-derived metabolites subsequently modified by microbial enzymes; Category III comprises microbiota-dependent host co-metabolites requiring sequential microbial and host metabolism; and Category IV comprises predominantly host- or tumor-derived mediators whose abundance, production, or signaling may be indirectly influenced by microbiota-associated metabolic networks. Not every category is represented by a separate metabolite row in this table. Primary BAs are host-derived precursors, whereas secondary and iso-bile acids generated through microbial modification are classified as Category II metabolites. Microbial Trp-derived indole metabolites are classified as Category I, whereas Kyn is predominantly generated through host- or tumor-associated IDO1/TDO2 metabolism and is therefore classified as a Category IV mediator. Polyamines are designated as source-dependent because both microbial and endogenous host pathways can contribute to their production and metabolism. Microbiota-derived B vitamins are considered Category I only when directly synthesized by

microorganisms; dietary B vitamins are exogenous nutrients and are not classified as microbiota-derived metabolites. Representative microbial and host sources are illustrative rather than exhaustive. Reported immune effects are context dependent and should not be interpreted as uniformly antitumor, protumor, anti-inflammatory, or pro-inflammatory across tissues, cell populations, and disease settings. Concentrations refer to the specific biological compartment or experimental system indicated in the table. Luminal, portal, peripheral-blood, tissue, intracellular, and in vitro concentrations are biologically non-equivalent and should not be compared directly. Likewise, descriptive terms such as physiological, elevated, low, medium, or high exposure do not represent universal concentration thresholds across metabolites or tumor contexts. μM denotes micromolar and mM denotes millimolar. Gene symbols are italicized, whereas protein and enzyme symbols are presented in roman type. Human gene symbols are written in uppercase letters, mouse gene symbols begin with an uppercase letter followed by lowercase letters, and bacterial gene symbols follow accepted organism-specific nomenclature. Bacterial genus and species names are italicized, whereas family, phylum, class, strain designations, and other higher taxonomic ranks are presented in roman type.

Table S2: Cell-specific effects of microbiota-associated metabolites: regulatory mechanisms, experimental models, concentration ranges, and evidence categories

Immune Cell Type	Metabolite	Representative producing taxa	Regulatory Effects	Direction of cellular effect	Molecular Mechanism	Research Model	Concentration Range of Metabolite Action	Evidence level and cancer relevance	References
cTreg	SCFAs (C2, C3, C4)	Firmicutes: Clostridium cluster XIV (ASF356/492), Roseburia, Eubacterium, *Faecalibacterium prausnitzii*; Bacteroidetes: Bacteroides fragilis, Prevotella	Increases cell number ($Foxp3^{+}$), promotes IL-10 secretion, enhances inhibitory capacity against Teff	↑ Expansion and suppressive function	1. FFAR2 (GPR43)-dependent signaling; 2. HDAC6/9 inhibition → histone acetylation; 3. Acetyl-CoA → mTOR activation	1. GF/SPF mice; 2. T cell-transfer colitis model in $Rag2^{-/-}$ mice; 3. Vancomycin-treated mice	1. Colonic lumen: proximal colon 130 mmol/kg, distal colon 80 mmol/kg, small intestine 15 mmol/kg; 2. Portal vein: C2 ≈ 250 µM, C3 20-200 µM, C4 15-65 µM; 3. Peripheral blood: C2 20-150 µM, C3 1-13 µM, C4 1-12 µM; 4. Drinking water treatment: 150 mM	Level I, direct human metabolite intervention in a non-cancer setting; Level IV, human observational evidence in IBD. Mechanistic preclinical evidence is also available, but direct evidence in tumor-associated cTregs remains limited	[1, 16, 17]

Th17 cell	SCFAs, including acetate, propionate, and butyrate	SCFA-producing bacteria, including Clostridium cluster XIV, Roseburia spp., Eubacterium spp., *Faecalibacterium prausnitzii*, Bacteroides spp., and Prevotella spp	Enhance Th17 effector function during bacterial or fungal infection but restrict excessive Th17 activation under homeostatic or inflammatory conditions	⇅ Context-dependent enhancement or suppression	HDAC inhibition and activation of the mTOR–S6K pathway, with the final effect depending on the immune and inflammatory context	Murine DSS-induced acute and chronic colitis models and peripheral-blood mononuclear cells from patients with inflammatory bowel disease	Colonic lumen: approximately 50–130 mmol/kg; portal vein: acetate approximately 250 μM, propionate 20–200 μM, and butyrate 15–65 μM; peripheral blood: acetate 20–150 μM, propionate 1–13 μM, and butyrate 1–12 μM; experimental drinking-water treatment: up to 150 mM	Levels II–III, mechanistic preclinical and ex vivo evidence in non-cancer inflammatory settings. Direct evidence in tumor-associated Th17 cells or patients with cancer remains limited	[16-18]
Th17 cell	3-oxolithocholic acid	3-oxoLCA-producing bacteria belonging to	Directly inhibits Th17-cell	↓ Differentiation	Direct binding to RORγt and inhibition of	In vitro differentiation of murine naïve	In vitro: 1–20 μM; murine cecal content:	Levels II–III, mechanistic preclinical	[19]

		Actinobacteria and Firmicutes, including Clostridium perfringens, as well as selected Bacteroides spp	differentiation and reduces the expression of the Th17 transcriptional program		RORγt-dependent Th17-associated gene expression	$CD4^+$ T cells, murine experimental autoimmune encephalomyelitis models, bacterial-colonization and dietary-feeding experiments, and murine intestinal-inflammation models	approximately 24 pmol/mg, corresponding to approximately 24 μM, after administration in a 0.3% weight/weight diet; a 1% dietary concentration was used in selected inflammatory models; average fecal concentration in patients with ulcerative colitis: approximately 23 pmol/mg	evidence; Level IV, human observational evidence in ulcerative colitis. Direct cancer-specific evidence remains lacking	
ILC3	SCFAs (C2, C3, C4)	Firmicutes: Clostridium cluster XIV,	Promotes IL-22 secretion,	↑ IL-22 production and protective	1. FFAR2 signaling → PI3K-AKT-ST	1. Murine C. rodentium/C. difficile	1. Colonic lumen: 50-130 mmol/kg; 2.	Level II, mechanistic preclinical	[16, 17]

		Roseburia; Bacteroidetes: Bacteroides spp.	enhances defense against extracellular bacterial infections (e.g., C. rodentium, C. difficile)	function	AT3 pathway activation; 2. HDAC inhibition → IL-22 promoter acetylation; 3. Potential AhR activation	infection model; 2. FFAR2$^{-/-}$ mice	Peripheral blood: C2 20-150 μM, C3 1-13 μM	evidence in non-cancer models; Level IV, human observational evidence in IBD. Direct cancer-specific evidence remains lacking	
ILC2	SCFAs (C2, C3, C4)	Same as ILC3	Inhibits IL-5/IL-13 secretion, reduces pulmonary allergic responses (e.g., Alternaria alternata-induced asthma)	↓ Type 2 cytokine production	1. Non-FFAR2-dependent: SCFA transport into cells → HDAC inhibition → histone acetylation; 2. Potential mTOR pathway inhibition	1. Murine pulmonary allergy model; 2. Murine parasite (helminth) infection model	1. Colonic lumen: 50-130 mmol/kg; 2. Lung: Increased C3/C4 concentration inhibits ILC2	Levels II–III, mechanistic preclinical evidence in non-cancer allergic and infectious models; Level IV, human observational evidence in asthma. Direct cancer-specific evidence remains lacking	[16, 17]

B Cell	SCFAs (C2, C3, C4)	Firmicutes: *Faecalibacterium prausnitzii*, Clostridium spp.; Bacteroidetes: Bacteroides spp.	1. Promotes differentiation of Germinal Center B (GCB) cells into plasma cells; 2. Increases IgG/IgA secretion, inhibits IgE production; 3. Enhances B cell metabolism (TCA cycle, fatty acid synthesis)	↑ Differentiation and antibody production	1. HDAC3 inhibition → activation of Aicda, Xbp1, Irf4 genes; 2. Acetyl-CoA → ATP production → mTOR activation; 3. FFAR2 signaling (partial effect)	1. Murine immune models (e.g., antigen immunization); 2. In vitro culture of human B cells	1. Peripheral blood: C2 20-150 μM, C3 1-13 μM, C4 1-12 μM; 2. In vitro culture: 0.1-1 mM (propionate/butyrate)	Levels II–III, mechanistic preclinical and in vitro evidence; Level IV, human observational evidence in non-cancer settings. Probiotic intervention provides indirect rather than metabolite-specific evidence. Direct cancer-specific evidence remains limited	[16, 17]
$CD8^+$ T Cell	SCFAs (mainly C4)	Firmicutes: Roseburia, Eubacterium, *Faecalibacterium prausnitzii*	1. Enhances cytotoxicity (IFN-γ, TNF-α secretion); 2. Promotes formation of	↑ Effector and memory function	1. HDAC inhibition → activation of Foxo1, Ppargc1a genes; 2. Acetyl-CoA → TCA cycle	1. Murine influenza virus infection model; 2. Murine tumor (melanoma, pancreatic cancer) model	1. Peripheral blood: C4 1-12 μM; 2. In vitro culture: 0.1-1 mM (butyrate)	Level II, cancer-relevant mechanistic preclinical evidence; Level IV, human observational	[16, 17]

			Tmem		→ ATP production			evidence in patients with cancer. No Level I direct metabolite-intervention evidence is currently established	
Treg (Systemic)	Polyamines (putrescine, spermidine, spermine)	1. Enterococcus faecalis; 2. *Bifidobacterium* animalis subsp. lactis; 3. Campylobacter jejuni; 4. Bacteroides thetaiotaomicron	1. Promotes Treg differentiation (Foxp3^{+}); 2. Inhibits inflammation (reduces TNF-α, IL-1β); 3. Alleviates autoimmune diseases (e.g., EAE)	↑ Differentiation and suppressive function	1. Autophagy activation (Atg5, FOXO3-dependent); 2. IDO1 activation → Trp metabolism; 3. eIF5A hypusination → mitochondrial OXPHOS	1. Murine EAE model; 2. Murine DSS colitis model; 3. PBMCs from patients with SLE or RA	1. In vitro culture: 2.5-15μM; 2. In vivo mouse drinking water: 30mM	Levels II–III, mechanistic preclinical and ex vivo evidence in non-cancer immune-disease models; Level IV, human observational evidence in SLE and RA. Direct cancer-specific evidence remains limited	[17, 20]
Th17 Cell (HCC Microenvironment)	SCFAs (C2, C3, C4)	Firmicutes: Oscillibacter; Bacteroidetes: Prevotella,	Reduces Th17 migration to tumors, decreases	↓ Tumor migration and IL-17 production	1. SCFAs promote intestinal Treg/Tr1 →	Murine subcutaneous HCC model (Hepa1-6 cells)	Specific concentration not defined, but fecal SCFA	Indirect microbiota-directed preclinical evidence. The	[21]

		Alistipes	IL-17 secretion → inhibits HCC angiogenesis		inhibit Th17 polarization; 2. Reduces CCR6 (migration receptor) expression on Th17		increases after probiotic intervention	intervention altered SCFA-associated microbial metabolism but did not establish direct metabolite-specific causality; direct Level I–III evidence for defined SCFA exposure in HCC-associated Th17 cells remains limited	
TAMs (pancreatic cancer)	ILA and IAA	Ligilactobacillus murinus and Limosilactobacillus reuteri	1. Promotes TAM immunosuppressive phenotype (increased expression of Arg1, IL-10, Ido1 genes, and Cyp1b1);	→ Immunosuppressive polarization	Activates AhR, regulates TAM transcriptome, inhibits inflammatory pathways, and promotes expression of immunosuppressive genes	1. Murine TAMs, human PBMC-derived macrophages, pancreatic cancer organoids; 2. Orthotopic PDAC model, iKPC model,	In vivo: 40 μg/kg (ILA/IAA) via gavage; In vitro: 250 μM IAA	Levels II–III, cancer-specific mechanistic preclinical and ex vivo evidence; Level IV, human observational evidence from PDAC	[22]

2. Inhibits intratumoral $CD8^+$T cell activation (reduces IFNγ, TNFα production and proliferation); 3. In vivo: Promotes pancreatic cancer growth; tumor enlargement in germ-free mice colonized with L. murinus; 4. In humans: High AHR expression in tumors of PDAC patients		and germ-free mice (C57BL/6); 3. Tumor samples and microbiota analysis from PDAC patients	specimens and clinical correlations. No Level I direct human metabolite-intervention evidence is available

			correlates with poor prognosis						
cDC precursors/cDC2	Butyrate	Butyrate-producing Firmicutes	Selectively inhibits cDC2 development and diminishes naïve T-cell priming	↓ Development and T-cell-priming capacity	HDAC3 inhibition, independently of GPCR signaling	Murine cDC-precursor differentiation and T-cell-priming assays	0.5 mM in vitro; 200 mM in drinking water in vivo	Levels II–III, mechanistic preclinical evidence in non-cancer experimental systems. Direct evidence in tumor-associated cDC2 populations or patients with cancer remains limited	[23]
NK Cell	SCFAs: acetate, propionate, and butyrate	Acetate: *Akkermansia muciniphila*, Bacteroides spp., *Bifidobacterium* spp. Propionate: Bacteroides	1. In primary human peripheral-blood NK cells, millimolar butyrate suppresses metabolic activation and	⇅ Context-dependent enhancement or restraint	1. Peripheral-blood NK cells: mTORC1–S6 and c-Myc signaling↓, glycolysis and oxidative phosphorylation	1. Primary human peripheral-blood NK cells stimulated with IL-12 and IL-15 and cocultured with K562 cells; 2. NK-92 and	Peripheral-blood NK cells: butyrate, 1–5 mM; NK-92 cells: acetate, 10 μM; propionate, 2 μM; butyrate, 2 μM;	Levels II–III, mechanistic preclinical and ex vivo evidence; cancer-specific evidence includes in vitro NK-92/MM.1S	[24-26]

spp., Phascolarctobacterium succinatutens, Dialister spp. Butyrate: *Faecalibacterium prausnitzii*, Roseburia spp., Eubacterium rectale	reduces the expression of activating receptors, cytokines, and cytolytic molecules, although direct killing of K562 cells is not significantly impaired; 2. In NK-92 cells, micromolar acetate, propionate, and butyrate modestly enhance cytotoxicity against MM.1S multiple-myeloma cells,	↓ under IL-12/IL-15 stimulation; 2. NK-92 cells: altered extracellular-vesicle and cytokine secretion, whereas the direct receptor or HDAC-dependent mechanism remains incompletely defined; 3. Liver-resident NK cells: GPR109A activation in Kupffer cells and hepatocytes → IL-18 production → NK-cell	MM.1S multiple-myeloma-cell coculture; 3. Mouse models and human liver samples examining liver-resident NK-cell maturation	liver-resident NK-cell study: tissue-dependent butyrate exposure without a directly comparable in vitro concentration threshold	experiments, whereas other findings derive from non-cancer or tissue-specific models. No Level I direct clinical metabolite-intervention evidence is available

increase extracellular-vesicle release, and reduce IL-10 secretion; 3. In liver-resident NK cells, butyrate indirectly supports maturation, mitochondrial activity, IFN-γ production, and degranulation through a tissue-dependent cytokine relay	maturation and metabolic activation

Neutrophil	Butyrate and propionate	Phylum Firmicutes (e.g., *Faecalibacterium prausnitzii*, Roseburia hominis), bacteria of order Clostridiales	1. Inhibits the secretion of pro-inflammatory cytokines (IL-6 and TNF-α) and chemokines (CCL3 and CXCL1); 2. Suppresses neutrophil migration and NET formation; 3. Alleviates intestinal mucosal inflammation	↓ Inflammatory activation, migration, and NET formation	1. Inhibits NF-κB signaling and reduces pro-inflammatory mediator expression; 2. Decreases the level of NET-associated citrullinated histone H3 (CitH3)	1. Peripheral-blood neutrophils from patients with IBD and healthy controls; 2. Murine DSS-induced colitis, germ-free, and antibiotic-treated mouse models	1. In vivo: Mice fed with drinking water containing 200 mM butyrate, or blood concentration elevated to above 0.45 mM via butyrate precursor (tributyrin); 2. Physiological concentration: 80-131 mM in colon, 79-375 μM in peripheral blood	Levels II–III, mechanistic preclinical and ex vivo evidence in non-cancer inflammatory models. Direct evidence in tumor-associated neutrophils or patients with cancer remains lacking	[27, 28]

Abbreviations: 3-oxoLCA, 3-oxolithocholic acid; acetyl-CoA, acetyl coenzyme A; AHR, aryl hydrocarbon receptor gene; AhR, aryl hydrocarbon receptor protein; Aicda, activation-induced cytidine deaminase gene; AKT, AKT serine/threonine kinase; Arg1, arginase 1 gene; ASF, altered Schaedler flora; Atg5, autophagy-related 5 gene; ATP, adenosine triphosphate; C2, acetate; C3, propionate; C4, butyrate; C57BL/6, C57BL/6 inbred mouse strain; CCL3, C-C motif chemokine ligand 3; CCR6, C-C motif chemokine receptor 6; cDC, conventional dendritic cell; cDC2, conventional type 2 dendritic cell; CD, cluster of differentiation; CitH3, citrullinated histone H3; c-Myc, MYC proto-oncogene protein; cTreg, colonic regulatory T cell; CXCL1, C-X-C motif chemokine ligand 1; Cyp1b1, cytochrome

P450 family 1 subfamily B member 1 gene; DSS, dextran sulfate sodium; EAE, experimental autoimmune encephalomyelitis; eIF5A, eukaryotic translation initiation factor 5A; FFAR2, free fatty acid receptor 2; FOXO3, forkhead box O3 protein; FOXP3, forkhead box P3 protein; Foxo1, forkhead box O1 gene; GCB, germinal center B cell; GF, germ-free; GPCR, G protein-coupled receptor; GPR43, G protein-coupled receptor 43; GPR109A, G protein-coupled receptor 109A; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; Hepa1-6, murine hepatoma cell line Hepa1-6; IAA, indole-3-acetic acid; IBD, inflammatory bowel disease; IDO1, indoleamine 2,3-dioxygenase 1 protein; Ido1, mouse indoleamine 2,3-dioxygenase 1 gene; IFN-γ, interferon-γ; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; ILA, indole-3-lactic acid; IL, interleukin; ILC2, group 2 innate lymphoid cell; ILC3, group 3 innate lymphoid cell; iKPC, inducible Kras/Trp53-driven pancreatic cancer model; Irf4, interferon regulatory factor 4 gene; K562, human chronic myeloid leukemia cell line K562; MM.1S, human multiple myeloma cell line MM.1S; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; NET, neutrophil extracellular trap; NF-κB, nuclear factor-κB; NK, natural killer; NK-92, human natural killer cell line 92; OXPHOS, oxidative phosphorylation; PBMCs, peripheral blood mononuclear cells; PDAC, pancreatic ductal adenocarcinoma; PI3K, phosphoinositide 3-kinase; Ppargc1a, peroxisome proliferator-activated receptor gamma coactivator 1-alpha gene; RA, rheumatoid arthritis; Rag2, recombination-activating gene 2; RORγt, retinoic acid-related orphan receptor γt; S6, ribosomal protein S6; S6K, ribosomal protein S6 kinase; SCFA, short-chain fatty acid; SCFAs, short-chain fatty acids; SLE, systemic lupus erythematosus; SPF, specific-pathogen-free; STAT3, signal transducer and activator of transcription 3; TAM, tumor-associated macrophage; TAMs, tumor-associated macrophages; TCA, tricarboxylic acid cycle; Teff, effector T cell; Th17, T helper 17 cell; Tmem, memory T cell; TNF-α, tumor necrosis factor-α; Tr1, type 1 regulatory T cell; Treg, regulatory T cell; Trp, tryptophan; Xbp1, X-box-binding protein 1 gene.

Notes: ↑ indicates an increase in the specified cellular abundance, differentiation, activity, expression, or function; ↓ indicates a decrease in the specified cellular abundance, differentiation, activity, expression, or function; ⇅ indicates context-dependent enhancement or suppression; → indicates a mechanistic sequence, downstream effect, or phenotypic transition; $^{+}$ or + indicates marker-positive cells; and $^{-/-}$ indicates homozygous gene deletion. Direction symbols refer specifically to the cellular phenotype or function stated in the corresponding entry and do not independently indicate an antitumor or protumor outcome. In vitro, ex vivo, and in vivo denote experiments performed outside a living organism, using cells or tissues removed from an organism, and within a living organism, respectively. Evidence levels are defined according to the causal strength and experimental context of the cited studies. Level I denotes direct human administration of a chemically defined metabolite with prospectively assessed clinical or pharmacodynamic outcomes. Level II denotes mechanistic in vivo evidence using defined metabolite exposure together with cell-, receptor-, transporter-, or pathway-specific perturbation. Level III denotes ex vivo or in vitro mechanistic evidence. Level IV denotes human observational evidence based on fecal, circulating, tissue, or microbiome–metabolome associations without direct metabolite intervention. Combined designations (e.g., Levels II–III) indicate that the cited evidence spans more than one experimental level. Evidence level and cancer relevance are evaluated separately; a higher evidence level obtained in a non-cancer setting does not constitute direct evidence of activity in tumor-associated immune cells or patients with cancer. Microbiota-directed

interventions, including probiotics, live bacterial products, dietary interventions, and fecal microbiota transplantation, are considered indirect evidence for a specific metabolite unless altered metabolite exposure and causal mediation are directly demonstrated. Non-cancer inflammatory, infectious, allergic, and autoimmune models are retained only when they provide mechanistic precedents for metabolite–immune-cell interactions and should not be interpreted as direct evidence of activity in tumor-associated immune cells or patients with cancer unless cancer-specific evidence is explicitly reported. Concentration ranges are specific to the indicated biological compartments and experimental models and should not be interpreted as directly equivalent to physiological exposure in tissues or the tumor microenvironment. μM, micromolar; mM, millimolar; μg/kg, micrograms per kilogram; mmol/kg, millimoles per kilogram; and pmol/mg, picomoles per milligram. Human gene symbols are italicized and written in uppercase letters, whereas mouse gene symbols are italicized and begin with an uppercase letter followed by lowercase letters. Protein symbols are presented in roman type. Bacterial genus and species names are italicized, whereas higher taxonomic ranks, strain designations, cell-line names, and mouse-strain designations are presented in roman type.

131 **Table S3. Reported concentrations of core microbiota-associated metabolites across physiological and tumor-associated biological compartments**

Metabolite	Biosynthetic origin	Biological compartment	Study context/model	Reported concentration/abundance	Analytical/quantification method	Major notes/limitations	References
Butyrate	Gut microbial fermentation of dietary fiber/carbohydrates	Feces	Human; colorectal cancer (CRC), n=14	3.79 ± 2.04 μg/mL, mean ± SD	Gas chromatography (GC)	Fecal butyrate was significantly lower in CRC than in non-CRC patients (6.81 ± 2.59 μg/mL, P=0.002); small cohort; the non-CRC group consisted mainly of patients with colitis/infection rather than healthy controls; fecal concentration does not directly represent systemic or intratumoral exposure	[29]
Butyrate	Gut microbial fermentation of fermentable dietary carbohydrates/fiber, including resistant	Feces	Human; healthy U.S. adults, n=313	9.49 ± 5.31 nmol/mg wet stool, mean ± SD	Gas chromatography–mass spectrometry (GC-MS); deuterated SCFA surrogates/internal standards and external	Represents physiological fecal exposure rather than tumor-associated exposure; fecal concentration reflects	[30]

	starch				standard curves	residual luminal SCFA and does not directly indicate circulating or intratumoral exposure; SCFA levels varied with host factors including age, BMI and sex	
Indole-3-propionic acid (IPA)	Gut microbial tryptophan metabolism; produced predominantly by *Clostridium sporogenes* and related anaerobic bacteria	Plasma	Human; fasting plasma from a population-based German cohort (Rhineland Study)	Median 1.156 µM; range 0–12.75 µM	Targeted UHPLC-ESI-MS/MS with triple-quadrupole MS and internal-standard-based quantification	Represents physiological systemic exposure rather than tumor-associated exposure; substantial interindividual variability; plasma and urine samples were derived from separate cohorts and were not paired within individuals	[31]
Indole-3-acetic acid (IAA)	Gut microbial tryptophan catabolism	Feces	Human; defined-diet cohort, n=44	44.64 ± 54.87 nmol/g, mean ± SEM; median 26.32 nmol/g, range	Targeted reverse-phase UHPLC-QTOF-MS; standard-curve	Represents physiological fecal exposure under a	[32]

				4.30–335.74 nmol/g	quantification with correction for recovery and matrix effects	controlled diet rather than tumor-associated exposure; substantial interindividual variability; fecal concentrations do not directly represent systemic or intratumoral exposure	
Indole-3-aldehyde (I3A)	Gut microbial tryptophan metabolism via the indole pathway	Feces	Human; colorectal cancer (CRC), n=39; healthy controls, n=28	CRC: 37.00 (13.00–63.00) nmol/L; healthy controls: 8.00 (4.25–15.50) nmol/L, median (Q1–Q3); $P < 0.001$	Targeted UPLC-MS/MS with authentic standards, calibration curves and MassLynx-based quantification	Fecal concentration does not directly represent intratumoral exposure; observational study cannot establish causality; diet was not standardized; original article abbreviates indole-3-aldehyde as IALD	[33]
Isoallolithocholic acid (isoalloLCA)	Gut microbial transformation of lithocholic acid (LCA), involving	Feces	Human; adults aged 45–75 years, n=138	0.05 (0.02–0.11) μmol/g, median (IQR)	Targeted LC/ESI-MS/MS using LCMS-8050 tandem MS;	Represents physiological fecal exposure rather than tumor-associated	[34]

	hydroxysteroid dehydrogenase- and reductase-dependent secondary bile acid metabolism				internal-standard-based bile acid quantification	exposure; isoalloLCA was detected in approximately 83% of stools; low-abundance metabolite; fecal level does not directly reflect circulating or intratumoral concentration	
Trimethylamine N-oxide (TMAO)	Gut microbial conversion of dietary choline, carnitine and related trimethylamine-containing substrates to TMA, followed by hepatic flavin monooxygenase-mediated oxidation to TMAO	Serum	Human; prospective PLCO nested case-control study; future incident CRC cases (n=761) vs individually matched controls (n=761)	Future CRC cases: 5.08 ± 5.25 μM; controls: 5.03 ± 7.46 μM, mean ± SD	Stable-isotope dilution targeted LC-MS/MS using triple-quadrupole MS	Prediagnostic baseline serum rather than samples collected after CRC diagnosis; TMAO was not associated with overall CRC risk, although higher TMAO was associated with distal CRC risk; circulating TMAO showed substantial interindividual and temporal variability	[35]

Deoxycholic acid (DCA)	Gut microbial 7α-dehydroxylation of cholic acid	Serum	Human; PLCO women who subsequently developed CRC, n=233	154.5 ± 203.6 ng/mL, mean ± SD	Targeted LC-MS/MS with isotope-labeled internal standards and calibration curves	Prediagnostic baseline serum rather than samples collected after CRC diagnosis; represents prospective CRC-associated circulating exposure; substantial interindividual variability	[36]
Deoxycholic acid (DCA)	Gut microbial 7α-dehydroxylation of cholic acid	Serum	Human; colorectal cancer, n=133	7.3 (2.6–39.8) ng/mL, median (IQR)	HPLC–LC-MS/MS using API 4000 QTRAP with authentic DCA standard	Serum DCA was markedly lower than in healthy controls [215.9 (103.8–456.5) ng/mL]; CRC and controls differed in age and sex, although adjusted and propensity-score-matched analyses confirmed significantly lower BA levels in CRC; circulating concentration does	[37]

						not directly represent fecal or intratumoral exposure	
Butyrate	Gut microbial fermentation of dietary carbohydrates/fiber	Serum	Human; surgically resected CRC patients, n=36	0.80 (0.51–8.47) μM, median (IQR)	Targeted HPLC-QqQ/MS with stable-isotope internal-standard calibration	Serum butyrate was significantly lower in CRC patients than in cancer-free controls [2.72 (2.11–3.70) μM, n=20; P=0.017]; small cohort; CRC and control groups differed in age, sex and BMI; circulating levels do not directly represent colonic luminal or intratumoral exposure	[38]
Lithocholic acid (LCA)	Gut microbial 7α-dehydroxylation of chenodeoxycholic acid	Serum	Human; newly diagnosed NSCLC, n=220	14.9 (5.5–30.2) nmol/L, median (IQR)	Validated LC-MS/MS using QTRAP 5500 with isotope-labeled internal standards	Serum LCA was lower than in matched healthy controls [19.9 (9.9–34.4) nmol/L, n=244; FDR-adjusted P=0.010]; serum exposure does not	[39]

						directly represent intratumoral concentration; dietary and underlying-disease confounding may remain	
Indole-3-acetic acid (IAA; 3-IAA)	Gut microbial tryptophan metabolism	Serum	Human; borderline resectable or locally advanced pancreatic ductal adenocarcinoma (PDAC), n=124; pretreatment	290 (203–417) ng/mL, median (IQR)	Targeted LC-MS/MS with deuterated 3-IAA-d5 internal standard and MRM detection	Pretreatment circulating exposure in non-metastatic PDAC; healthy controls showed 377 (303–464) ng/mL; 3-IAA was not significantly associated with chemotherapy response or overall survival; circulating concentration does not directly represent intratumoral exposure	[40]
Trimethylamine N-oxide (TMAO)	Gut microbial conversion of dietary choline and L-carnitine	Serum	Human; gastric cancer (GC), n=105;	GC: 3.02 (2.06–4.99) nmol/mL; controls: 2.65 (2.00–3.66) nmol/mL,	UPLC-MS/MS with internal-standard-based quantification	Pretreatment circulating exposure; overall TMAO	[41]

	to trimethylamine (TMA), followed by hepatic oxidation to TMAO		controls without high-risk gastric lesions, n=83	median (IQR)		difference between GC and controls was not statistically significant (P=0.064); sex-specific differences were observed, with significantly higher TMAO in male GC patients; circulating concentration does not directly represent intratumoral exposure	
Isolithocholic acid (isoLCA)	Gut microbial epimerization of lithocholic acid through hydroxysteroid dehydrogenase-mediated metabolism	Feces	Human; healthy adults aged 19–22 years, n=14	0.41 ± 0.39 μmol/g, mean ± SD	LC/MS using LCMS-8050 tandem mass spectrometry with internal standards	Represents physiological fecal exposure; value obtained without strong alkaline hydrolysis (Method A), therefore mainly reflects directly extractable fecal isoLCA and may underestimate esterified/saponifiable isoLCA; fecal	[42]

						concentration does not directly represent circulating or intratumoral exposure	
Acetate	Gut microbial fermentation of dietary carbohydrates	Feces	Human; healthy adults, n=61; longitudinal repeated fecal sampling	16.11 (8.29–26.06) μmol/g feces, median (IQR); range 0.85–76.07 μmol/g	3-Nitrophenylhydrazine derivatization followed by UPLC-QTOF-MS; isotope-labeled internal standards and external standard calibration	Represents physiological fecal exposure rather than tumor-associated exposure; repeated measurements showed substantial day-to-day variability; fecal concentration does not directly represent circulating or intratumoral exposure	[43]
Propionate	Gut microbial fermentation of non-digestible dietary carbohydrates/fiber	Feces	Human; medically confirmed healthy Thai adults, n=157	9.61 ± 5.09 μmol/g, median ± MAD	Derivatization-free GC-TOFMS using propionic-d5 acid as internal standard and matrix-matched calibration	Represents physiological fecal exposure rather than tumor-associated exposure; cohort was predominantly young adults and dietary intake was	[44]

						self-reported; fecal concentrations do not directly represent circulating or intratumoral exposure	
Indole-lactic acid (ILA)	Gut microbial tryptophan metabolism	Plasma	Human; hepatocellular carcinoma (HCC), n=41; healthy controls, n=96	HCC: 1.06 (0.36) μmol/L; healthy controls: 0.91 (0.42) μmol/L, mean (SD); P=0.038	Stable-isotope-dilution targeted LC-MS/MS with isotope-labeled internal standards	Plasma ILA was significantly higher in HCC than in healthy controls; 61% of HCC patients had underlying cirrhosis, and ILA was also higher in HCC with cirrhosis than HCC without cirrhosis; relatively small HCC cohort; circulating concentration does not directly represent intratumoral exposure	[45]

trans-3-Indoleacrylic acid (IDA)	Gut microbial tryptophan metabolism via the indole pathway	Feces	Human; healthy controls, n=36; IBS-D patients, n=42	Healthy controls: 0.0082 ± 0.0042 ng/mg; IBS-D: 0.0072 ± 0.0097 ng/mg, mean ± SD; P=0.660	Targeted HPLC-MS/MS using UHPLC separation and QTRAP 6500+ MS; isotope-labeled internal standard used during extraction	Provides human fecal absolute concentration; difference between healthy controls and IBS-D was not significant; IBS-D is a non-neoplastic gastrointestinal disorder, so this row represents physiological/non-tumor gastrointestinal exposure rather than tumor-associated exposure; fecal levels do not directly represent circulating or intratumoral exposure	[46]

Abbreviations: BMI, body mass index; CRC, colorectal cancer; DCA, deoxycholic acid; FDR, false discovery rate; GC-MS, gas chromatography – mass spectrometry; GC-TOFMS, gas chromatography – time-of-flight mass spectrometry; HCC, hepatocellular carcinoma; HPLC, high-performance liquid chromatography; I3A, indole-3-aldehyde; IAA, indole-3-acetic acid; IBS-D, diarrhea-predominant irritable bowel syndrome; IDA, trans-3-indoleacrylic acid; ILA, indole-lactic acid; IPA, indole-3-propionic acid; IQR, interquartile range; isoalloLCA, isoallolithocholic acid; isoLCA, isolithocholic acid; LC-MS/MS, liquid chromatography – tandem mass spectrometry; LCA, lithocholic acid; MAD, median absolute deviation; MRM, multiple reaction monitoring; MS, mass spectrometry; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer

Screening Trial; QTOF-MS, quadrupole time-of-flight mass spectrometry; QTRAP, quadrupole linear ion trap; SD, standard deviation; SEM, standard error of the mean; SCFA, short-chain fatty acid; TMA, trimethylamine; TMAO, trimethylamine N-oxide; UHPLC, ultra-high-performance liquid chromatography; UPLC, ultra-performance liquid chromatography.

Note: Human absolute quantitative data remain limited for several compartments, particularly portal circulation, tumor tissue, and intracellular exposure. These compartments are therefore not represented by in vitro treatment concentrations, which are not considered equivalent to physiological or pathological metabolite concentrations.

Table S4: Microbiota-associated metabolites across selected cancer contexts: regulatory mechanisms, treatment associations, and evidence levels

Cancer type	Key metabolite(s)	Immunoregulatory mechanism	Treatment-associated effect	Evidence level and causal strength	References
CRC	SCFAs, including acetate, propionate, and butyrate	1. Butyrate inhibits HDAC activity and suppresses CRC cell proliferation through epigenetic and cell-cycle regulation; 2. Low-concentration butyrate promotes Treg differentiation via GPR43 to exert anti-inflammatory effects; 3. High-concentration butyrate induces T-bet expression and IFN-γ secretion in $CD4^+$ T cells, enhancing $CD8^+$ T cell cytotoxicity; 4. Propionate promotes proteasomal degradation of EHMT2, reduces H3K9me2 levels, and induces tumor cell apoptosis	1. Butyrate supplementation enhances oxaliplatin-induced tumor regression; 2. Synergizes with 5-fluorouracil (5-FU) to improve 5-FU sensitivity by restoring SMCT1 expression; 3. Enhances IL-12 signaling in the tumor TME to promote IFN-γ secretion by $CD8^+$ T cells	Retrospective associations between fecal SCFA profiles and CRC outcomes, together with preclinical treatment studies; direct clinical evidence for butyrate-based combination therapy remains limited	[47, 48]

Gastric Cancer	BAs: DCA, LCA, UDCA, glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA)	1. DCA may promote myeloid-derived suppressor cell expansion and M2 macrophage polarization through FXR-associated signaling, thereby contributing to immune suppression; however, tumor-cell studies have also reported that DCA inhibits epithelial–mesenchymal transition by increasing MUC2 expression and reducing Snail and MMP9 expression; 2. LCA may activate NF-κB/STAT3-associated inflammatory signaling, promote the release of IL-6 and TNF-α, and exacerbate chronic inflammation; 3. UDCA: Inhibits NF-κB activation, reduces inflammatory responses, and protects gastric mucosa; 4. Elevated GCA/GCDCA levels are associated with inflammatory immune-cell infiltration	1. UDCA: Enhances efficacy of 5-FU and cisplatin chemotherapy, and reduces chemotherapy-induced mucositis; 2. Elevated DCA/LCA is associated with chemotherapy resistance in GC; 3. Elevated serum GCA/GCDCA levels are associated with a lower immunotherapy response rate	1. Retrospective cohort studies: Analyzed associations between serum BA profiles and prognosis in GC patients; 2. Case-control studies: Compared BA concentrations between GC patients and healthy individuals; 3. Preclinical studies: Mouse models of UDCA-adjuvanted chemotherapy	[49-54]
CRC	IDA	A single experimental study reported activation of the AhR–ALDH1A3–FSP1–CoQ10 axis and enhanced ferroptosis resistance in CRC cells	May contribute to CRC progression by limiting ferroptosis; implications for treatment sensitivity require further validation	Preclinical experimental evidence from a single study; independent replication remains unavailable	[55]

Melanoma, breast cancer, and CRC	IPA	Epigenetically regulates *Tcf7* in progenitor exhausted T cells and supports T-cell stemness and sustained antitumor activity	Enhances immunotherapy efficacy in multiple preclinical cancer models	Pan-cancer preclinical experimental study	[56]
Pancreatic cancer	Indole-3-acetic acid (IAA)	Enhances chemotherapy-induced tumor-cell killing and modulates $CD8^+$ T-cell and neutrophil responses	Enhances chemotherapy efficacy	Experimental and translational study	[57]
Lung Cancer (NSCLC/SCLC)	SCFAs, including acetate, propionate, and butyrate	1. Inhibits HDAC to regulate inflammatory gene transcription; 2. Activates G protein-coupled receptors (GPR41/GPR43/GPR109A) to promote Treg differentiation and enhance $CD8^+$T cell cytotoxicity; 3. Regulates IL-10/TGF-β secretion by alveolar macrophages (AMs) to maintain pulmonary immune homeostasis	1. Probiotic interventions that may alter SCFA production have been evaluated as adjuncts to cisplatin-based chemotherapy; Fecal SCFA levels have been associated with responses and adverse events during PD-1/PD-L1 blockade; 3. Preclinical studies suggest potential protection against radiotherapy-induced lung injury	Indirect microbiota-directed and Level IV observational human evidence; SCFA-specific therapeutic causality has not been established	[58-60]
Breast Cancer	SCFAs, particularly butyrate	1. Butyrate acts as an HDAC inhibitor to induce breast cancer cell apoptosis; 2. Maintains intestinal barrier and reduces bacterial translocation; 3. Regulates innate/adaptive immunity	1. Sodium butyrate enhances chemosensitivity of TNBC and synergizes with anti-cancer drugs; 2. Improves chemotherapy-related intestinal mucositis	Levels II–III preclinical mechanistic evidence together with Level IV human observational evidence; direct quantitative evidence linking circulating SCFA exposure to specific intratumoral immune-cell	[61, 62]

				responses remains limited	
Breast Cancer	TMAO	1. Activates PERK to induce GSDME-mediated pyroptosis in breast cancer cells; 2. Promotes IL-1β/IL-18 release and enhances $CD8^+$ T cell infiltration/cytotoxicity	1. Combined with anti-PD-1 antibodies significantly inhibits tumor growth; 2. Higher plasma TMAO levels were associated with immunotherapy response	Level II preclinical mechanistic evidence and Level IV human observational evidence from TNBC cohorts, including plasma TMAO analyses; no direct clinical TMAO-intervention evidence is available	[63]
Prostate Cancer	SCFAs, including acetate, propionate, and butyrate	Experimental evidence indicates that SCFAs can influence prostate-cancer progression through tumor-associated IGF1–MAPK/PI3K signaling and may additionally modify tumor–immune interactions through cell-specific metabolic and inflammatory pathways	In experimental models, reduced microbial SCFA production following antibiotic treatment was associated with suppressed prostate-tumor growth, whereas SCFA supplementation restored circulating IGF1 and reversed this effect, supporting a context-dependent tumor-promoting role for SCFAs through the IGF1–MAPK/PI3K axis	Preclinical mechanistic evidence with limited human observational support; direct clinical intervention evidence for defined SCFA exposure remains lacking	[64-66]
Melanoma	IAA	Intratumoral L. reuteri-derived IAA activates AhR in $CD8^+$ T cells and	Was reported to enhance immune-checkpoint inhibitor	Preclinical mechanistic and translational evidence; direct	[67]

		increases IFN-γ and granzyme B production	efficacy in preclinical melanoma models	clinical metabolite-intervention evidence is lacking	
Melanoma	Butyrate and propionate	Higher systemic SCFA exposure was associated with regulatory immune features and reduced antitumor immune activity during CTLA-4 blockade	Higher circulating butyrate and propionate concentrations were associated with reduced clinical benefit from CTLA-4 blockade, indicating that SCFA–immunotherapy associations may differ according to checkpoint target and treatment context	Level IV human observational evidence; treatment-specific association without direct metabolite intervention	[68, 69]
Multiple myeloma	Acetate, propionate, and butyrate	SCFA pretreatment of NK-92 cells increases cytotoxicity against MM.1S cells, enhances extracellular-vesicle release, and reduces NK-cell IL-10 secretion; the direct molecular pathway remains incompletely defined	Micromolar SCFA exposure modestly enhanced NK-92-cell cytotoxicity against MM.1S cells in vitro, suggesting a potential context-dependent interaction with NK-cell antitumor activity	Level III in vitro mechanistic evidence; no direct in vivo or clinical metabolite-intervention evidence is available	[24]
MASLD-associated hepatocellular carcinoma	Butyrate-enriched SCFA profile	Associated with expansion of total Tregs and IL-10^{+} effector Tregs and attenuation of cytotoxic CD8^{+} T-cell responses; potential involvement of FOXP3-associated chromatin remodeling and antigen-presenting-cell-mediated retinoic-acid signaling	May be associated with an immunosuppressive hepatic immune state; its effect on immunotherapy responsiveness has not been directly established	Levels III–IV ex vivo and human observational evidence; causal intrahepatic concentration thresholds and the dominant responding cell population remain unresolved	[70]

Abbreviations: 5-FU, 5-fluorouracil; AhR, aryl hydrocarbon receptor; ALDH1A3, aldehyde dehydrogenase 1 family member A3; AMs, alveolar macrophages; BA, bile acid; BAs, bile acids; CD, cluster of differentiation; CoQ10, coenzyme Q10; CRC, colorectal cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DCA, deoxycholic acid; EHMT2, euchromatic histone lysine methyltransferase 2; FOXP3, forkhead box P3; FSP1, ferroptosis suppressor protein 1; FXR, farnesoid X receptor; GC, gastric cancer; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GPR41, G protein-coupled receptor 41; GPR43, G protein-coupled receptor 43; GPR109A, G protein-coupled receptor 109A; GSDME, gasdermin E; H3K9me2, histone H3 lysine 9 dimethylation; HDAC, histone deacetylase; IAA, indole-3-acetic acid; IDA, trans-3-indoleacrylic acid; IFN-γ, interferon-γ; IGF1, insulin-like growth factor 1; IL, interleukin; IPA, indole-3-propionic acid; LCA, lithocholic acid; M2, alternatively activated macrophage phenotype; MAPK, mitogen-activated protein kinase; MASLD, metabolic dysfunction-associated steatotic liver disease; MM.1S, human multiple myeloma cell line MM.1S; MMP9, matrix metalloproteinase 9; MUC2, mucin 2; NF-κB, nuclear factor-κB; NK, natural killer; NK-92, human natural killer cell line 92; NSCLC, non-small-cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; PERK, protein kinase R-like endoplasmic reticulum kinase; PI3K, phosphoinositide 3-kinase; SCFA, short-chain fatty acid; SCFAs, short-chain fatty acids; SCLC, small-cell lung cancer; SMCT1, sodium-coupled monocarboxylate transporter 1; STAT3, signal transducer and activator of transcription 3; T-bet, T-box transcription factor TBX21; Tcf7, mouse transcription factor 7 gene; TGF-β, transforming growth factor-β; TMAO, trimethylamine N-oxide; TME, tumor microenvironment; TNBC, triple-negative breast cancer; TNF-α, tumor necrosis factor-α; Treg, regulatory T cell; Tregs, regulatory T cells; UDCA, ursodeoxycholic acid.

Notes: Evidence levels are defined according to the causal strength and experimental context of the cited studies. Level I denotes direct human administration of a chemically defined metabolite with prospectively assessed clinical or pharmacodynamic outcomes. Level II denotes mechanistic in vivo evidence using defined metabolite exposure together with cell-, receptor-, transporter-, or pathway-specific perturbation. Level III denotes ex vivo or in vitro mechanistic evidence. Level IV denotes human observational evidence based on fecal, circulating, tissue, or microbiome–metabolome associations without direct metabolite intervention. Combined designations (e.g., Levels II–III or Levels III–IV) indicate that the cited evidence spans more than one experimental level. Associations identified in retrospective or prospective human cohorts should not be interpreted as evidence of causality or direct metabolite-specific therapeutic efficacy unless metabolite exposure and causal mediation are experimentally established. Microbiota-directed interventions provide indirect evidence for the activity of a specific metabolite unless altered metabolite exposure and causal mediation are directly demonstrated. Treatment-associated effects summarize findings within the specified cancer, metabolite, biological compartment, and treatment context and should not be extrapolated to other cancer types or therapeutic settings. Opposing effects reported for the same or related metabolites across cancers or treatment settings are retained to reflect context-dependent functional variation rather than interpreted as biological inconsistency. IAA is used consistently for indole-3-acetic acid, although this metabolite was termed 3-IAA in the original pancreatic-cancer study. IAA, IDA, and IPA denote chemically distinct Trp-derived metabolites and should not be used interchangeably. Gene symbols are italicized, whereas protein symbols are presented in roman type. Human gene symbols are written in uppercase letters, whereas mouse gene symbols begin with an uppercase letter followed by lowercase letters.

Bacterial genus and species names are italicized, whereas strain, cell-line, and mouse-strain designations are presented in roman type.

174 **Table S5: Translational strategies involving microbiota-associated metabolites and microbiota-directed interventions in cancer**
175 **immunotherapy**

Therapeutic Strategy Type	Specific Method	Core Mechanism	Reported or potential advantages	Limitations	Evidence stage, study model, and causal inference	References
Biomarker	Serum/fecal SCFAs, including acetate, propionate, and butyrate	1. Associations between circulating or fecal SCFAs and immune-checkpoint blockade outcomes are treatment and compartment dependent; higher SCFA exposure has been associated with favorable outcomes in selected anti-PD-1 settings, whereas higher circulating butyrate and propionate concentrations have been associated with reduced clinical benefit during CTLA-4 blockade in melanoma; 2. SCFAs can modulate $CD8^+$ T-cell activity and cytokine production through GPCR- and HDAC-associated pathways; 3. Fecal or	1. Enables minimally invasive assessment using fecal or circulating samples; 2. May support longitudinal monitoring of metabolite-associated changes during treatment; 3. Potentially applicable to context-specific response monitoring across selected cancers, although cross-cancer	1. Significantly affected by diet (dietary fiber intake) with poor stability; 2. Large individual metabolic differences, lack of unified reference thresholds; 3. Unable to distinguish causal relationships (metabolite changes vs. treatment response)	1. Animal models: MC38 colon cancer mice, B16 melanoma mice, lung cancer mice; 2. Clinical observations: Elevated serum SCFAs in NSCLC patients and ICBT responders	[48, 59, 71]

		circulating SCFA abundance may reflect microbiota-associated metabolic activity but does not directly establish intratumoral metabolite exposure or causal immune regulation	generalizability and clinically validated thresholds remain unestablished			
Biomarker	Serum TMAO	1. Higher circulating TMAO levels have been associated with ICB response in selected cohorts, whereas preclinical studies indicate that TMAO can activate type I interferon signaling and induce tumor-cell pyroptosis, thereby enhancing $CD8^+$ T-cell activity; 2. Reflects gut microbiota choline metabolic function ($CutC^+$ bacteria), associated with M1 macrophage infiltration in TME; 3. Enhances $CD8^+$T cell-mediated anti-tumor immunity in TNBC	1. Circulating TMAO is analytically measurable and may provide a candidate metabolite-associated biomarker in selected cancer settings; 2. Associations between circulating TMAO and clinical outcomes are cancer dependent and should not be generalized across tumor types; 3. Preclinical studies	1. High concentration may promote tumorigenesis via inflammation induction in CRC, exhibiting functional paradox; 2. Detection relies on mass spectrometry with high cost; 3. Significantly influenced by red meat/choline intake	1. Animal models: PDAC mice, TNBC mice, CRC mice; 2. Clinical observations: Positive correlation between fecal TMA-producing bacteria abundance and anti-PD-1 response in PDAC patients; negative correlation	[48, 71, 72]

			suggest that TMAO may modify ICB responsiveness in defined tumor contexts		between serum TMAO and survival rate in CRC patients	
Biomarker	Serum Trp Metabolites 3-HAA, Kyn/Trp ratio	1. Decreased 3-HAA concentration correlates with prolonged PFS in NSCLC patients receiving anti-PD-1 therapy, reducing Treg proliferation and DC secretion of TGF-β; 2. Kyn/Trp ratio reflects IDO1 activity; high ratio indicates ICBT resistance; 3. Combined with PD-L1 expression improves accuracy of ICBT efficacy prediction	1. Directly linked to TME immunosuppressive status with clear mechanism; 2. Enhances predictive value when combined with PD-L1 expression; 3. Non-invasive serum detection with good reproducibility	1. Regulated by both host IDO1 and microbiota, leading to complex interpretation; 2. Validated only in specific cancers (NSCLC, CRC), with unproven universality	1. Animal models: Lung cancer mice, CRC mice; 2. Clinical observations: NSCLC patients, CRC patients	[48, 59]

Biomarker	Fecal/Tissue Abundance of Parabacteroides distasonis and Bacteroides vulgatus	1. Abundance of both bacteria significantly higher in anti-PD-1 responders than non-responders with NSCLC; 2. Positively correlated with $CD8^+$T cell activity (IFN-γ/TNF-α secretion) and negatively correlated with Treg proportion; 3. Reflects the degree of gut microbiota remodeling toward a "responder-type" profile	1. Non-invasive detection (fecal samples) with high specificity; 2. Predicts anti-PD-1 efficacy in NSCLC patients; 3. Combined detection with metabolites (valeric acid, Kyn/Trp) improves predictive accuracy	1. Validated only in Asian NSCLC populations, with unclear racial differences; 2. Influenced by diet (e.g., dietary fiber), requiring validation of stability; 3. Cannot be used as a standalone predictive indicator, requiring combination with metabolites or immune markers	1. Animal models: Germ-free mouse FMT model (fecal transplantation from NSCLC non-responders, increased bacterial abundance after combination with GPs + anti-PD-1); 2. Clinical observations: NSCLC patients (n=16, responders n=10)	[73, 74]

Biomarker	Fecal Abundance of Streptococcus anginosus and Streptococcus constellatus	1. Abundance of both bacteria significantly increased in gastric cancer patients, positively correlated with gastric mucosal inflammation and carcinogenesis severity; 2. Serves as an indicator for early screening and progression monitoring of gastric cancer	1. Non-invasive (fecal samples), suitable for screening high-risk populations of gastric cancer; 2. Highly associated with gastric precancerous lesions (intestinal metaplasia, atrophic gastritis)	1. Susceptible to oral microbiota contamination, requiring strict quality control in sample processing; 2. Universality unvalidated in other digestive system tumors	Clinical observations: Gastric cancer patients (multicenter cohort);	[74]
Biomarker	Serum Valeric Acid, Kyn/Trp Ratio	1. Increased valeric acid activates $CD8^+$T cells (inhibits HDAC, upregulates IL-12Rβ2); 2. Decreased Kyn/Trp ratio reflects inhibited IDO activity, reducing Treg differentiation and enhancing Th1 immunity; 3. Combined use predicts anti-PD-1 response and irAEs risk	1. Convenient serum detection for dynamic monitoring of treatment response; 2. Directly linked to TME immune status ($CD8^+$T cell/Treg balance)	1. Valeric acid concentration significantly affected by gut microbiota metabolism with large individual differences; 2. Kyn/Trp ratio regulated by both host IDO1 and microbiota, leading to complex interpretation	1. Animal models: LLC lung cancer mice, B16 melanoma mice; 2. Clinical observations: NSCLC patients	[73, 75]

Metabolite Supplementation/Intervention	Direct Inosine Supplementation	1. Binds to T cell adenosine A_2A receptor (A_2AR), promotes Th1 differentiation and $CD8^+$T cell IFN-γ secretion under IFN-γ/IL-12 co-stimulation; 2. Provides alternative carbon source for $CD8^+$T cells/CAR-T cells under glucose restriction to maintain effector function; 3. Inhibits UBA6 to stabilize tumor cell MHC-I and enhance immunogenicity	1. Long in vivo half-life (>15 h), substantially exceeding that of adenosine (~10 s); 2. Preclinical studies have reported activity in combination with ICBT, CAR-T cells, and adoptive T-cell therapy; 3. Randomized phase II evaluation has not demonstrated a statistically significant improvement in the primary objective-response-rate endpoint	1. The immunological effect of inosine may depend on the presence of appropriate co-stimulatory signals and treatment context; 2. The randomized Phase II study did not demonstrate a statistically significant improvement in its primary objective-response-rate endpoint; 3. Independent multicenter validation and metabolite exposure–response analyses remain required	Preclinical tumor models and a randomized, open-label Phase II clinical trial (NCT05809336) involving 172 patients with advanced solid tumors receiving oral inosine combined with PD-1 or PD-L1 blockade	[71, 72]
Microbiota-directed intervention	Live bacterial-product or probiotic intervention (Clostridium butyricum CBM588 and mixed Bifidobacterium strains)	1. CBM588 is a butyrate-producing live bacterial product that can alter gut microbial and metabolic profiles; preclinical studies	1. Early-phase studies have reported exploratory efficacy signals in selected	1. Limited efficacy in non-antibiotic users, restricting applicable populations; 2. Butyrate production affected by	1. Animal models: MC38 colon cancer mice, B16 melanoma	[59, 71]

		support SCFA-associated immune mechanisms, whereas clinical effects observed with CBM588 cannot be attributed specifically to butyrate without direct demonstration of metabolite mediation; 2. Mixed *Bifidobacterium* strains (B. bifidum/B. longum, etc.) activate DC STING/IFN-I pathway and enhance anti-CD47 therapy efficacy; 3. Regulates gut microbiota, increasing abundance of beneficial bacteria (Lactobacilli, Bifidobacteria)	ICB-treated populations; 2. Clinical safety and immune-related adverse-event outcomes require further validation; 3. Oral administration provides a feasible route for clinical evaluation	intestinal environment with poor stability; 3. Long-term effects on gut microbiota balance remain unknown	mice; 2. Clinical Phase I/II: mRCC patients, NSCLC patients	
Metabolite Supplementation/Intervention	Prebiotic Supplementation (Dietary Fiber, Inulin, Pectin)	1. Promotes gut microbiota fermentation to produce SCFAs (butyrate, propionate), activates GPR43 to enhance $CD8^+$T cell activity; 2. Pectin enriches Lachnospiraceae/Ruminococcaceae, increases SCFA production, and improves anti-PD-1 response in CRC	1. Widely available dietary sources, including whole grains and vegetables, with relatively low implementation cost; 2. May support intestinal-barrier	1. Slow onset (requires microbiota metabolic conversion), unsuitable for acute conditions; 2. High-fiber diet may increase intestinal burden in CRC patients; 3. Individual microbiota differences lead to variable SCFA	1. Animal models: APC^Min/+ CRC mice, lung cancer mice; 2. Clinical observations: CRC patients, NSCLC	[48, 59]

		patients; 3. Inulin regulates microbiota, reduces secondary bile acid (DCA) production, and inhibits CRC progression	function and microbiota-associated metabolic modulation; 3. Cancer-specific efficacy and long-term safety require prospective clinical validation	production rates	patients	
Microbiota-directed intervention	Fecal microbiota transplantation (FMT)	1. FMT reshapes recipient gut-microbiota composition and may transfer microbiota-associated features linked to ICB responsiveness; 2. FMT can alter multiple microbial communities, metabolites, and host immune pathways simultaneously, but observed clinical effects cannot generally be attributed to a specific metabolite without demonstrating altered metabolite exposure and causal mediation; 3. FMT has also been investigated for microbiota-associated	1. Enables broad microbiota remodeling in treatment-refractory settings; 2. Early-phase clinical studies have demonstrated feasibility and reported response signals in selected melanoma and NSCLC populations; 3. May provide a platform for investigating microbiota-associat	1. Strict donor screening (exclusion of pathogens, metabolic abnormalities required); 2. Poor post-transplant microbiota stability with potential recurrence of resistance; 3. Risk of transmitting pathogenic or multidrug-resistant organisms and causing serious infections; 4. Clinical effects cannot be attributed to a single microbiota-associated metabolite unless altered metabolite exposure and	1. Animal models: Germ-free tumor-bearing mice, NSCLC mice; 2. Clinical Phase I/II: Melanoma patients, NSCLC patients	[48, 59, 71]

		modulation of ICB-related gastrointestinal toxicity	ed effects on both treatment response and gastrointestinal toxicity	causal mediation are directly demonstrated		
Dietary/prebiotic microbiota-directed intervention	Oral ginseng polysaccharides	1. Acts as prebiotic to enrich intestinal Muribaculum, P. distasonis, B. vulgatus, and promote valeric acid production; 2. Reduces gut microbiota-mediated Trp decomposition, decreases Kyn production, and inhibits IDO activity; 3. Enhances intestinal barrier function (upregulates epithelial protective genes such as CLCA3, TFF3)	1. Orally administered polysaccharides with potential for microbiota-directed modulation; 2. Preclinical studies suggest concurrent effects on microbiota composition, metabolite profiles, and immune regulation; 3. Reversal of antibiotic-associate d anti-PD-1 resistance remains supported primarily by preclinical evidence	1. Efficacy dependent on intestinal microbiota status, with limited effects in patients with severe microbiota dysbiosis; 2. Large individual differences in valeric acid production, requiring personalized dose adjustment; 3. Not yet entered clinical trials	Animal models: LLC lung cancer mice, HuPD-1 knock-in lung cancer mice, B16 melanoma mice	[73]

Metabolite Supplementation/Intervention	Supplementation with Anacardic Acid (Metabolite of Bacteroides caccae)	1. Activates macrophage MAPK pathway (phosphorylates ERK1/2, JNK) to promote M1 polarization; 2. Induces neutrophil extracellular trap (NETs) formation and recruits tumor-infiltrating immune cells (macrophages, NK cells, T cells); 3. Induces breast cancer cell apoptosis and enhances CTLA-4 inhibitor efficacy	1. Natural metabolite evaluated in preclinical tumor models; 2. Experimental studies suggest effects on both innate and adaptive immune compartments; 3. Antitumor activity has been reported in selected melanoma and breast-cancer models, but clinical evidence remains unavailable	1. Validated only in preliminary experiments, lacking large-scale animal experimental data; 2. Dependent on intestinal microbiota composition, requiring B. caccae colonization; 3. Not yet entered clinical research	1. Animal models: Breast cancer mouse model; 2. Clinical observations: Fecal metabolome detection in melanoma patients (62-fold concentration increase in responders)	[75]
Combination Therapy	Metabolite + ICBT (e.g., TMAO + anti-PD-1, Inosine + anti-CTLA-4)	1. TMAO activates macrophage IFN-I pathway, synergizes with anti-PD-1 to enhance $CD8^{+}$T cell infiltration in PDAC; 2. Inosine combined with anti-CTLA-4 promotes	1. Preclinical studies have reported synergistic immune effects in selected tumor models; 2. Whether metabolite	1. TMAO may promote tumorigenesis in CRC, requiring strict indication screening; 2. Difficult to precisely control metabolite doses (easily affected by	1. Animal models: PDAC mice, TNBC mice, HCC mice; 2. Clinical observations:	[59, 71, 72]

		intratumoral Th1 cell accumulation and enhances anti-melanoma efficacy; 3. Secondary bile acids (UDCA/LCA) combined with anti-PD-1 regulate DC function and improve response in HCC patients	combinations reduce ICB dose or immune-related adverse events remains clinically unestablished; 3. Their ability to overcome clinical ICB resistance requires prospective validation	microbiota); 3. Combined mechanisms not fully elucidated	Positive correlation between TMAO and anti-PD-1 response in PDAC patients	
Host metabolic-pathway-targeted combination therapy	IDO1 inhibitor (epacadostat or navoximod) + anti-PD-1	1. Inhibits IDO1 activity, reduces Trp metabolism to kynurenine, and relieves Treg-mediated immunosuppression; 2. Synergizes with anti-PD-1 to activate $CD8^+$T cells, enhance tumor infiltration and IFN-γ secretion; 3. Reduces Kyn/Trp ratio and reverses primary ICBT resistance	1. Specifically relieves metabolism-mediated immunosuppression with clear mechanism; 2. Showed preliminary activity in early-phase studies, but clinical benefit has not been consistently confirmed; 3. Oral	1. Phase III trial (ECHO-301) failed in melanoma, with efficacy limited by tumor type; 2. May increase gastrointestinal toxicity (e.g., diarrhea, nausea); 3. Requires patient screening combined with PD-L1 expression	1. Animal models: Lung cancer mice, CRC mice; 2. Clinical Phase I/II: NSCLC patients, CRC patients	[48, 59]

			IDO inhibitors with convenient administration			
Metabolite- or pathway-directed combination therapy	Butyrate or pharmacological HDAC inhibition (e.g., entinostat) combined with anti-PD-1	1. Butyrate inhibits HDAC, enhances $CD8^+$T cell T-bet/Eomes expression, and maintains effector function; 2. Entinostat reduces MDSC infiltration, synergizes with anti-PD-1 to activate $CD8^+$T cells in lung cancer patients; 3. Regulates tumor cell epigenetics, increases PD-L1 expression, and enhances ICBT sensitivity	1. Can modulate both T-cell and tumor-cell pathways in experimental models; 2. Butyrate and pharmacological HDAC inhibitors are mechanistically related but non-equivalent interventions, and a clinical safety advantage of butyrate has not been established; 3. Reversal of ICB resistance remains context dependent and requires clinical validation	1. Synthetic HDAC inhibitors (entinostat) cause hematological toxicity (e.g., thrombocytopenia); 2. Rapid in vivo metabolism of butyrate, requiring continuous supplementation; 3. Limited efficacy in tumors with low HDAC expression	1. Animal models: MC38 colon cancer mice, lung cancer mice; 2. Clinical Phase I/II: NSCLC patients	[59, 71]

Combination Therapy	GPs + anti-PD-1 Monoclonal Antibody (e.g., Pembrolizumab/Nivolumab)	1. GPs reshape gut microbiota (increases P. distasonis/B. vulgatus), elevates valeric acid to activate $CD8^+$T cells; 2. Reduces Kyn/Trp ratio, inhibits Treg differentiation, and relieves immunosuppression; 3. Enhances intratumoral $CD8^+$T cell infiltration (increased IFN-γ/TNF-α/GZMB secretion)	1. Preclinical and observational studies suggest potentially favorable microbiota-associated immune changes during anti-PD-1 therapy; 2. Reliable conversion of clinical nonresponders and reduction of immune-related adverse events have not been established; 3. Generalizability across tumor types requires further validation	1. Antibiotic use impairs efficacy (destroys microbiota); 2. Efficacy in *H. pylori* infection-positive patients requires further validation; 3. Limited clinical data	1. Animal models: Germ-free mouse FMT model (fecal transplantation from NSCLC non-responders, 75.2% increase in tumor inhibition rate after combination therapy), LLC lung cancer mice; 2. Clinical observations: NSCLC patients	[73]
Engineered microbial combination therapy	Engineered Escherichia coli Nissle 1917 strain SYNB1891 producing cyclic di-AMP + atezolizumab	1. Following intratumoral administration, SYNB1891 produces CDA under hypoxic conditions, activating STING signaling in tumor-resident	1. Designed for tumor-localized delivery and reduced systemic exposure; 2.	1. Only entered Phase I clinical trial, long-term safety unclear; 2. Tumor colonization efficiency affected by TME	1. Animal models: B16-F10 melanoma mice; 2.	[75]

		phagocytic antigen-presenting cells; 2. Induces IFN-I production by intratumoral monocytes/DCs and promotes $CD8^+$T cell activation; 3. Synergizes with anti-PD-L1 to relieve T cell exhaustion and enhance tumor infiltration	Activates STING-associated antitumor immune signaling within the tumor microenvironment; 3. Preclinical antitumor activity has been reported across selected solid-tumor models, while long-term clinical efficacy and biosafety remain under evaluation	hypoxia level; 3. Requires combination with ICIs, with limited efficacy when used alone	Clinical Phase I: Patients with advanced solid tumors	
Microbiota-directed combination therapy	Live biotherapeutic product MRx0518 + pembrolizumab	1. MRx0518 activates TLR5/NF-κB pathway via flagellin; 2. Promotes DC maturation and IL-12 secretion, enhancing Th1 immunity; 3. Synergizes with pembrolizumab to improve $CD8^+$T cell activity	1. Oral administration with high patient compliance; 2. Applicable to NSCLC, RCC, melanoma, etc.; 3. Early-phase clinical safety and efficacy remain under	1. Phase I/II clinical efficacy remains to be validated; 2. Efficacy may depend on host TLR5 signaling; 3. Treatment effects may also vary according to the baseline gut-microbiota background	Clinical Phase I/II: NSCLC, RCC, bladder cancer patients	[75]

			evaluation			
Dietary microbiota-modulating strategy	High-salt diet + low-dose anti-PD-1 antibody	1. High-salt diet increases intestinal permeability and promotes *Bifidobacterium* colonization in tumors; 2. Enhances NK cell activation (increased NCR expression), synergizes with anti-PD-1 to improve $CD8^+$T cell infiltration	1. Low-cost and experimentally tractable dietary intervention; 2. Enhances anti-PD-1 activity in mouse models	1. Contraindicated in patients with hypertension/nephropathy; 2. Validated only in mouse models, no clinical data; 3. Long-term high-salt intake may damage intestinal barrier	Animal models: Melanoma mice	[75]
Combination Therapy	Probiotic Lactobacillus kefiranofaciens ZW18 + anti-PD-1 Monoclonal Antibody	1. ZW18 regulates gut microbiota and increases SCFA (butyrate) production; 2. Activates $CD8^+$T cells (upregulates IFN-γ/GZMB) and inhibits Treg differentiation; 3. Synergizes with anti-PD-1 to enhance tumor suppression	1. Probiotic intervention with microbiota-modulating potential; 2. Preclinical studies suggest effects on gut microbial composition and antitumor immune responses; 3. Clinical efficacy and safety across cancer types remain to be established	1. Validated only in mouse models, no clinical data; 2. Efficacy in *H. pylori* infection-positive gastric cancer patients requires validation; 3. Significantly affected by antibiotics	Animal models: Gastric cancer mouse model	[74]

176 **Abbreviations:** 5-FU, 5-fluorouracil; AhR, aryl hydrocarbon receptor; ALDH1A3, aldehyde dehydrogenase 1 family member A3; AMs, alveolar

macrophages; BA, bile acid; BAs, bile acids; CD, cluster of differentiation; CoQ10, coenzyme Q10; CRC, colorectal cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DCA, deoxycholic acid; EHMT2, euchromatic histone lysine methyltransferase 2; FOXP3, forkhead box P3; FSP1, ferroptosis suppressor protein 1; FXR, farnesoid X receptor; GC, gastric cancer; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GPR41, G protein-coupled receptor 41; GPR43, G protein-coupled receptor 43; GPR109A, G protein-coupled receptor 109A; GSDME, gasdermin E; H3K9me2, histone H3 lysine 9 dimethylation; HDAC, histone deacetylase; IAA, indole-3-acetic acid; IDA, trans-3-indoleacrylic acid; IFN-γ, interferon-γ; IGF1, insulin-like growth factor 1; IL, interleukin; IPA, indole-3-propionic acid; LCA, lithocholic acid; M2, alternatively activated macrophage phenotype; MAPK, mitogen-activated protein kinase; MASLD, metabolic dysfunction-associated steatotic liver disease; MM.1S, human multiple myeloma cell line MM.1S; MMP9, matrix metalloproteinase 9; MUC2, mucin 2; NF-κB, nuclear factor-κB; NK, natural killer; NK-92, human natural killer cell line 92; NSCLC, non-small-cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; PERK, protein kinase R-like endoplasmic reticulum kinase; PI3K, phosphoinositide 3-kinase; SCFA, short-chain fatty acid; SCFAs, short-chain fatty acids; SCLC, small-cell lung cancer; SMCT1, sodium-coupled monocarboxylate transporter 1; STAT3, signal transducer and activator of transcription 3; T-bet, T-box transcription factor TBX21; Tcf7, mouse transcription factor 7 gene; TGF-β, transforming growth factor-β; TMAO, trimethylamine N-oxide; TME, tumor microenvironment; TNBC, triple-negative breast cancer; TNF-α, tumor necrosis factor-α; Treg, regulatory T cell; Tregs, regulatory T cells; UDCA, ursodeoxycholic acid.

Notes: Evidence levels are defined according to the causal strength and experimental context of the cited studies. Level I denotes direct human administration of a chemically defined metabolite with prospectively assessed clinical or pharmacodynamic outcomes. Level II denotes mechanistic in vivo evidence using defined metabolite exposure together with cell-, receptor-, transporter-, or pathway-specific perturbation. Level III denotes ex vivo or in vitro mechanistic evidence. Level IV denotes human observational evidence based on fecal, circulating, tissue, or microbiome–metabolome associations without direct metabolite intervention. Combined designations (e.g., Levels II–III or Levels III–IV) indicate that the cited evidence spans more than one experimental level. Associations identified in retrospective or prospective human cohorts should not be interpreted as evidence of causality or direct metabolite-specific therapeutic efficacy unless metabolite exposure and causal mediation are experimentally established. Microbiota-directed interventions provide indirect evidence for the activity of a specific metabolite unless altered metabolite exposure and causal mediation are directly demonstrated. Treatment-associated effects summarize findings within the specified cancer, metabolite, biological compartment, and treatment context and should not be extrapolated to other cancer types or therapeutic settings. Opposing effects reported for the same or related metabolites across cancers or treatment settings are retained to reflect context-dependent functional variation rather than interpreted as biological inconsistency. IAA is used consistently for indole-3-acetic acid, although this metabolite was termed 3-IAA in the original pancreatic-cancer study. IAA, IDA, and IPA denote chemically distinct Trp-derived metabolites and should not be used interchangeably. Gene symbols are italicized, whereas protein symbols are presented in roman type. Human gene symbols are written in uppercase

letters, whereas mouse gene symbols begin with an uppercase letter followed by lowercase letters. Bacterial genus and species names are italicized, whereas strain, cell-line, and mouse-strain designations are presented in roman type. Reported advantages and therapeutic effects should be interpreted according to the evidence stage indicated in each row. Findings derived from preclinical models or observational cohorts should not be interpreted as established clinical efficacy or safety, and microbiota-directed interventions do not constitute direct metabolite-specific evidence unless altered metabolite exposure and causal mediation are demonstrated.

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