nature communications

Review article

https://doi.org/10.1038/s41467-026-72178-1

Microbiota-derived metabolites as modulators of cancer immunotherapy response

Received: 11 August 2025

Accepted: 28 March 2026

Published online: 21 April 2026

Check for updates

Catherine Toner-Bartelds[1,2,4], Iris L. Mimpen[1,2,4], Miguel Parra-Martinez[1,2], Boudewijn M. T. Burgering[2,3] & Emile E. Voest[1,2]

The microbiome is a key regulator of host homeostasis and immune activity, in part through the production of metabolites. These microbiota-derived metabolites can modulate both the innate and adaptive immune system, as well as directly target tumour cells, thereby regulating anti-tumour immunity and response to immunotherapy. Here, we describe the current mechanistic knowledge on how these metabolites exert their effects and outline the methodologies used to detect and assess these metabolites. Finally, we summarize microbiota-targeted therapies capable of improving microbial functionality to ultimately enhance immunotherapy responses and improve patient survival.

Cancer treatment outcomes have improved greatly since the advent of immunotherapy. In particular, immune checkpoint blockade (ICB) has shown great promise across a wide variety of cancer types[1]. The most assessed ICB therapies target programmed cell death protein 1 (PD1), programmed death ligand-1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). However, response rates are still limited, with 45–60% in melanoma and 15–30% in other solid tumours[2]. This highlights the need to further understand factors influencing ICB responsiveness, thereby aiming to optimise treatment efficacy.

One newly identified modulator of response to immunotherapy is the microbiome, which has recently been included as one of the hallmarks of cancer[3]. The microbiome is a complex community of microorganisms, including bacteria, viruses, and fungi, that reside in the body and live in a mutualistic relationship with the host. A first hint for a role of the microbiome in ICB response came from studies showing that antibiotic treatment prior to or during ICB treatment results in a worsened response[4,5]. Additionally, transplanting faeces from ICB-responding and non-responding patients to mice reproduced the corresponding treatment response patterns[6]. Subsequently, several studies demonstrated that the composition of the microbiome was significantly different between ICB responders and non-responders[6–8]. Furthermore, microbiome-targeting therapies, such as faecal microbiota transfer (FMT) and probiotics, have shown promise in improving ICB responses[9–15]. However, the mechanisms by which the microbiome, as well as microbiome-targeting therapies, including FMT, modulate ICB response are still largely unknown. Additionally, bacterial strains considered to be important for response are inconsistent across studies[9], which is not surprising given that the composition of the microbiome varies greatly even across healthy individuals[16,17]. Therefore, it is crucial to further understand how the microbiome modulates anti-tumour immunity, as this will enable the development of new microbiota-targeting strategies that can complement ICB and support clinical translation.

In contrast to microbiome composition, microbial functional capacity (i.e. its metabolic output) remains highly stable across individuals[16]. Importantly, the microbiome can produce a diverse range of metabolites which function as nutrient sources for the host, but also as signalling molecules capable of inducing downstream effects, including modulating the immune system[18]. These immunomodulatory functions of microbiota-derived metabolites could therefore provide an interesting mechanistic link between the microbiome and its influence on ICB outcome. Indeed, several studies have now identified microbiota-derived metabolites that can influence anti-tumour immunity and thereby affect ICB efficacy. This highlights the need to look beyond single species associations and investigate the impact of microbial metabolic activity on immunotherapy outcomes.

[1]Division of Molecular Oncology & Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands. [2]Oncode Institute, The Netherlands Cancer Institute, Amsterdam, The Netherlands. [3]Molecular Cancer Research, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, The Netherlands. [4]These authors contributed equally: Catherine Toner-Bartelds, Iris L. Mimpen. e-mail: e.voest@nki.nl

Here, we provide a comprehensive overview of microbiota-derived metabolites that have been shown to modulate anti-tumour immunity and response to immunotherapy. We focus on their effects on different compartments of the immune system and describe the mechanisms through which they exert their function. Additionally, we discuss methodologies and model systems used in these studies and outline their challenges, as well as opportunities to guide future research. Finally, we address the translational potential of this research through examining potential microbiota-derived interventions, which ultimately can be used to boost the therapeutic response to immunotherapies.

The microbiome and the immune system

The microbiome forms a dynamic ecosystem, spanning a broad spectrum of taxonomic classifications, starting from the phylum down to the species level, and the abundance of the various species varies greatly across individuals. Although these taxonomic classifications are helpful for mapping microbial diversity, microbes within the same taxonomic group can exert different functional capacities. This emphasises that analyses based solely on microbial composition may overlook critical aspects of microbiome function. Additionally, in contrast to the variation in community composition, the functional capacity of the microbiome is highly conserved across individuals[16,19], highlighting the importance of metabolic activity of the microbiome. The number of microbial genes exceeds that of the human genome by approximately 100-fold, with over 19,000 metabolite gene clusters identified across microbial genomes[20]. This enables the production of a wide range of metabolites, a broad overview of which is provided in Table 1, with details on different mechanisms of production outlined in Box 1. Many of these metabolites can interact either directly or indirectly with the host immune system, thereby playing a key role in shaping human immune responses.

In the context of cancer, microbes in the gut can produce metabolites, which can act on immune cells locally in the gut, or at distant sites, such as the tumour. This microbiota-mediated immune modulation may result in altered anti-tumour immunity and therefore affect response to ICB. Additionally, microbes also reside in the tumour, where they might be able to produce metabolites and subsequently alter the immune context within the tumour. Below, we provide an overview (summarised in Table 2) of how microbiota-derived metabolites impact response to immunotherapy, focusing on their effects on different compartments of the immune system and their suggested mechanisms.

Innate immunity

The innate immune system, which consists of cells such as macrophages and natural killer (NK) cells, is the first line of defence against invading pathogens, initiating an immediate immune response. It is therefore unsurprising that microbiota-derived metabolites can directly impact the phenotype and function of these cells (Fig. 1). In the context of immunotherapy, microbiota-derived metabolites, such as indoles[21] and trimethylamine *N*-oxide (TMAO)[22], have been shown to affect anti-tumour immunity through their influence on tumour-associated macrophages (TAMs). For example, Mirji et al. studied the role of the gut microbiome in pancreatic ductal adenocarcinoma (PDAC) growth by treating mice with antibiotics, which resulted in a significant increase in tumour burden. To investigate the underlying mechanism, the authors performed untargeted LC-MS/MS

Table 1 | Overview of metabolite production by the gut microbiome

Class	Metabolites	Origin	Microbe production method
Short-chain fatty acids (SCFAs)	Butyrate, propionate, pentanoate, formate	Produced by microbial fermentation of dietary fibres and complex carbohydrates.	Diet-microbiota
Indoles and tryptophan metabolites	Indole-3-acetic acid (IAA), Indole-3-aldehyde (I3A), Indole-lactic acid (ILA), Indole-3-propionic acid (IPA)	Derived from dietary tryptophan, metabolised by gut bacteria.	Diet-microbiota Cross-feeding
Trimethylamine derivatives	Trimethylamine N-oxide (TMAO)	Derived from TMA, which is generated by gut microbes from dietary choline, carnitine, or phosphatidylcholine.	Microbiota-host
Amino acids	L-Serine, L-Arginine.	Derived from dietary protein and host biosynthesis but may be modulated by microbial uptake and metabolism.	Diet-microbiota
Amino acid derivatives	Urocanic acid (UCA), Phenylacetylglutamine (PAGln)	Derived from amino acid metabolism by gut microbes (histidine and phenylalanine, for UCA and PAGln, respectively).	UCA = host-microbiota PAGln = microbiota-host
Secondary bile acids	Ursodeoxycholic acid (UDCA), 3-oxo-Δ4,6-lithocholic acid	Generated from primary bile acids by microbial deconjugation and transformation.	Host-microbiota
Nucleoside derivatives	Inosine	Derived from the breakdown of purine nucleosides.	Host-microbiota De novo microbial synthesis Cross-feeding
Polyunsaturated fatty acids	Docosahexaenoic acid (DHA)	Derived from the diet but may be modified or influenced by microbial metabolism.	Diet-microbiota
Coenzymes and vitamins	Coenzyme A (CoA), Pantothenate (vitamin B5), Riboflavin derivatives (5-OP-RU).	De novo microbial synthesis	De novo microbial synthesis
TCA cycle metabolite	Succinic acid	Intermediate in the TCA cycle derived from succinyl-CoA.	Diet-microbiota
Other microbial-derived bioactive compounds	Urolithin A (UA), Trigonelline, Phytosphingosine, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP)	Other microbial-derived bioactive compounds have effects on the host.	UA = Diet-microbiota Trigonelline = diet/host-microbiota, however, is largely plant-derived. Phytosphingosine = De novo microbial synthesis HMBPP = De novo microbial synthesis

SCFAs short chain fatty acids, *IAA* indole-3-acetic acid, *I3A* indole-3-aldehyde, *ILA* indole-lactic acid, *IPA* indole-3-propionic acid, *TMAO* trimethylamine N-oxide, *TMA* trimethylamine, *UCA* urocanic acid, *PAGln* phenylacetylglutamine, *DHA* docosahexaenoic acid, *CoA* coenzyme A, *5-OP-RU* 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil, *UA* urolithin A, *HMBPP* (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate.

BOX 1

Directions of microbiota-derived metabolite production

Microbiota-derived metabolites are diverse in their nature and function, and the mechanisms through which they are produced rely on an intricate network of interactions. These interactions depend not only on a dynamic exchange between the diet, the host, and the microbiome, but also on cross-feeding relationships between microbes themselves. Here, we provide an overview of the types of interactions that may occur during microbial metabolite production.

Diet-microbiota: dietary intake can greatly influence the composition of the microbiome, as well as the metabolites produced. Dietary components, such as carbohydrates, can act as substrates for these microbiota-derived metabolites. For instance, SCFAs, including butyrate, acetate, and propionate, are produced through bacterial fermentation of dietary fibres. Dietary fibre is broken down by bacteria to form simple sugars, which are metabolised, for example, through glycolysis, to produce pyruvate and acetyl-CoA[109]. The production of butyrate then relies on the microbial enzymes butyryl CoA:acetate CoA transferase and butyryl kinase, while the production of propionate occurs through the succinate, acrylate or propanediol pathways. Acetate, the most abundant SCFA, is produced through acetyl-CoA by or through the Wood-Ljungdahl pathway. Additional SCFAs include formate and pentanoate. Further examples of diet-derived microbial metabolites include urolithin A (UA), derived from the breakdown of ellagitannins[110]; indoles, derived from the catabolism of tryptophan through the action of the enzyme tryptophanase[111]; and succinic acid, a central intermediate of carbohydrate fermentation.

Host-microbiota: in other cases, metabolite production requires an initial transformation by the host. This is demonstrated by the sequential host-mediated processing of cholesterol to primary bile acids, followed by secondary bile acid production by the microbiota. These primary bile acids, such as cholic acid, are synthesised in the liver from cholesterol through the action of enzymes including cholesterol 7α-hydroxylase and further conjugated with glycine or taurine[112]. Primary bile acids are then released into the intestine and transformed to secondary bile acids (deoxycholic acid, lithocholic acid (LCA) and UDCA) by enzymes such as bile salt hydrolases and hydroxysteroid dehydrogenases from the intestinal microbiota[112].

Microbiota-host: in some circumstances, sequential processing of substrates first by microbes and then by the host is required. A well-characterised example of this is TMAO. Diet-derived choline and L-carnitine are first converted to trimethylamine (TMA) by the gut microbiota through genes such as CutC/D[113] and the *gbu* operon[114]. Further transport to and oxidation in the liver by flavin-containing monooxygenases is then required to generate TMAO. The metabolite PAGln is a further example of this type of interaction, whereby phenylalanine is first converted to phenylacetic acid by microbes. Phenylacetic acid is then conjugated with glutamine in the liver[115].

De novo production. Metabolites may also be directly generated by microbes using simple precursors. Examples of metabolites produced de novo include pantothenate, or riboflavin derivatives such as 5-OP-RU and HMBPP. Pantothenate can be derived from the diet or produced de novo by bacteria through the action of enzymes such as pantothenate synthetase[116]. Riboflavin derivatives are produced through the riboflavin synthesis pathway, with 5-OP-RU being formed through the condensation of 5-A-RU and methylglyoxal. HMBPP is produced as an intermediate in the non-mevalonate (MEP) pathway, a bacterial-specific pathway.

Microbe-microbe cross-feeding: generation of microbiota-derived metabolites can also occur in a stepwise fashion, relying on the cross-feeding interactions between bacteria. This is exemplified by the cross-feeding interaction between *Lactobacillus johnsonii* and *Clostridium sporogenes* to produce the downstream tryptophan metabolite indole-3-propionic acid (IPA). Firstly, tryptophan is converted to indole-3-lactic acid (ILA) by *L. johnsonii*. ILA is then further converted to IPA by *C. sporogenes*[54]. This interaction underscores the collaboration which can occur between microbes to facilitate metabolite production.

Drug-microbe interactions: drugs used during the treatment of diseases can have a direct impact on microbiome composition and thereby the types of metabolites produced. In some instances, drugs themselves can be directly converted to new metabolites by the microbiome[117]. For example, in vitro treatment of a faecal-derived bacterial community with trifluridine, an anti-cancer drug, resulted in the deglycosylation of trifluridine to the inactive form trifluorothymine[117]. In other cases, drug treatment may induce shifts in the microbiome, which directly impacts metabolite production. For example, following the cessation of antibiotic administration, a bloom in *Enterocloster* species can occur resulting in large shifts in bile acid production[118]. An additional example of this is the tyrosine kinase inhibitor (TKI) induced increase in *Muribaculum gordoncarteri* abundance, leading to increased urocanic acid (UCA) production[68].

Considering these interactions and their combined effects is crucial when defining the relevance of the microbiome in microbiome-immune system interactions. A complex network of interactions is required for the generation of different microbiota-derived metabolites, both between the diet, the host, and its microbiota, as well as among microbes within the microbiome. Careful interpretation of where these metabolites are derived from is therefore essential to prevent oversimplification of the role of the microbiome in ICB response and to enable the development of effective strategies for microbiota-targeting treatments.

metabolomics on serum from these PDAC-bearing mice and observed a depletion of TMAO (microbiota-host, Box 1) upon antibiotic treatment[22]. They subsequently showed that this metabolite can skew TAMs and dendritic cells (DCs) towards an immunostimulatory phenotype by activating a type I interferon (IFN) response, which in turn drives CD8 T cell activation and enhances anti-PD1 and anti-TIM3 efficacy in a PDAC mouse model. Finally, increased abundance of bacteria capable of producing this metabolite and expression of CutC, the enzyme required for its production, was associated with improved responses in patients treated with anti-PD1. In contrast to this metabolite-induced macrophage activation, Hezaveh et al. identified a role for the microbiome, in particular *Lactobacillus murinus* and *Lactobacillus reuteri*, in worsening ICB response through inducing immunosuppressive TAMs in PDAC-bearing mice[21]. The authors observed hampered anti-tumour immunity upon aryl hydrocarbon receptor (AhR) activity in TAMs, which was improved after treatment with *Lactobacillus*-targeted antibiotics. Mechanistically, metabolomics on bacterial supernatant, as well as faeces of mice receiving these bacteria via oral gavage, revealed tryptophan-derived indoles (diet-microbiota, cross-feeding, Box 1), including indole-3-acetic acid (IAA) and indole-lactic acid (ILA), to be capable of activating AhR expressed on TAMs, thereby generating an immunosuppressive phenotype that hampered CD8 T cell activity. Accordingly, removal of tryptophan from the diet of PDAC-bearing mice led to decreased AhR activity on TAMs and increased the number of activated CD8 T cells in the

https://doi.org/10.1038/s41467-026-72178-1

Table 2 | Overview of metabolites influencing anti-tumour immunity and ICB response

Metabolite type	Metabolite	Cancer type	Model (discovery)	Method used	Bacteria involved	Model (validation)	Immune compartment/ tumour	Immune effect	Gut vs tumour microbiome	Treatment type	Study
SCFA	Butyrate, Propionate	Melanoma	Human	Targeted metabolomics (Stool and serum)		Mice (in vivo)	Innate (DCs)	Immunosuppressive	Gut	Anti CTLA-4	Coutzac et al.[24]
	Pentanoate, Butyrate	Melanoma and pancreatic cancer	Targeted selection (focus on a subset of bacteria)	Targeted metabolomics (Bacterial supernatant)	*Megasphaera massiliensis*	Mice (in vivo); human (in vitro)	Adaptive (CD8 T cells and CAR-T cells)	Immunostimulatory	Gut	Adoptive T cell therapy	Luu et al.[51]
	Butyrate	MSS-CRC	Mixed: human (bacteria) and mice (metabolite)	Untargeted metabolomics (Bacterial supernatant and stool) and Targeted metabolomics (stool and tumour)	*F. nucleatum*	Mice (in vivo); human (in vitro)	Adaptive (CD8 T cells)	Immunostimulatory	Tumour/Gut	Anti PD-1	Wang et al.[52]
	Butyrate	NSCLC	Human	Targeted metabolomics (stool and serum)		Mice (in vivo); human (in vitro)	Adaptive (CD8 T cells) and innate-like (Vγ9Vδ2 T cells)	Immunostimulatory	Gut	Anti PD-1	Zhu et al.[53]
	Butyrate	CRC	Mixed: human (bacteria) and mice (metabolite)	Untargeted metabolomics (bacterial supernatant and stool)	*Roseburia intestinalis*	Mice (in vivo)	Adaptive (CD8 T cells)	Immunostimulatory	Gut	Anti PD-1	Kang et al.[56]
	Formate	Melanoma	Mice	Untargeted metabolomics (stool) and targeted metabolomics (caecum, serum, and tumour)		Mice (in vivo; in vitro); human (in patient validation of serum formate)	Adaptive (CD8 T cells)	Immunostimulatory	Gut	Anti PD-L1	Phelps et al.[46]
Indoles and tryptophan metabolites	Indoles (ILA and IAA)	PDAC	Mice	Targeted metabolomics (bacterial supernatant)	*Lactobacillus murinus* and *Lactobacillus reuteri*	Mice (in vivo; in vitro)	Innate (TAMs)	Immunosuppressive	Gut	Anti PD-L1	Hezaveh et al.[21]
	I3A	Melanoma	Targeted selection (focus on probiotic bacteria) in mice	Targeted metabolomics (bacterial supernatant)	*L. reutreri*	Mice (in vivo); human (in patient validation of serum I3A)	Adaptive (CD8 T cells)	Immunostimulatory	Tumour	Anti PD-L1/ PD-1	Bender et al.[47]
	IPA	Melanoma, breast cancer, CRC.	Mice	Untargeted metabolomics (serum and bacterial supernatant)	*Lactobacillus johnsonii* and *Clostridium sporogenes*	Mice (in vivo); human (in vitro)	Adaptive (CD8 T cells)	Immunostimulatory	Gut	Anti PD-1	Jia et al.[54]
	IPA	B-cell lymphoma	Human	Targeted metabolomics (serum)	*Akkermansia* species	Mice (in vivo)	Adaptive (CAR-T cells)	Immunostimulatory	Gut	Adoptive T cell therapy	Marcos-Kovandzic et al.[48]

https://doi.org/10.1038/s41467-026-72178-1

Table 2 (continued) | Overview of metabolites influencing anti-tumour immunity and ICB response

Metabolite type	Metabolite	Cancer type	Model (discovery)	Method used	Bacteria involved	Model (validation)	Immune compartment/ tumour	Immune effect	Gut vs tumour microbiome	Treatment type	Study
Trimethylamine derivatives	TMAO	Breast cancer (TNBC)	Human	Untargeted metabolomics (tumour)		Mice (in vivo; in vitro); human (in patient validation of serum TMAO; in vitro)	Tumour cells	Immunostimulatory	Tumour	Anti PD-1/ PD-L1	Wang et al.[70]
	TMAO	PDAC	Mice	Targeted and untargeted metabolomics (serum)		Mice (in vivo); human (clinical cohort follow-up; in vitro)	Innate (TAMs)	Immunostimulatory	Gut	Anti PD-1/ anti-TIM3	Mirji et al.[22]
Amino acids	L-serine	Melanoma	Mixed: human (bacteria) and mice (metabolite)	Untargeted metabolomics (stool)	*Eubacterium rectale*	Mice (in vivo; vitro)	Innate (NK cells)	Immunosuppressive	Gut	Anti PD-1	Liu et al.[26]
	L-arginine	CRC	Mice	Untargeted metabolomics (stool)	*Clostridiaceae*	Mice (in vivo); human (in patient validation of serum arginine levels)	Tumour cells	Immunostimulatory	Gut	Anti PD-L1 + MEK inhibitor	Zhang et al.[65]
Amino acid derivatives	PAGln	CRC	Human	Untargeted metabolomics (stool)		Mice (in vivo)	Adaptive (CD8 T cells)	Immunosuppressive	Gut	Anti PD-1/ PD-L1	Zhu et al.[57]
	UCA	CRC	Human and mice	Untargeted metabolomics (stool and serum/plasma)	*Muribaculum*	Mice (in vivo); human (in patient validation of serum and faecal UCA)	Endothelial cells	Immunostimulatory	Gut	TKI + Anti PD-1	Zhang et al.[68]
Secondary bile acids	Secondary bile acids (LCA, MCA)	HCC and liver metastasis	Mice	Targeted metabolomics (liver)	*Clostridium* species	Mice (in vivo)	Innate-like (NKT cells)	Immunosuppressive	Gut	Not linked to ICB efficacy	Ma et al.[42]
	Novel secondary bile acids (3-oxo-Δ4-LCA)	Bladder cancer	Targeted selection (direct focus on bile acids) in humans and mice	Targeted BA metabolomics (stool and serum)		Mice (in vivo); human (in vitro)	Adaptive (CD8 T cells)	Immunostimulatory	Gut	Anti PD-1	Jin et al.[49]
	UDCA	CRC, melanoma and lung cancer	Targeted selection (direct focus on UDCA)	No metabolomics		Mice (in vivo); human (clinical cohort follow-up; in vitro)	Adaptive (CD8 T cells and Tregs)	Immunostimulatory	Gut	Anti PD-1	Shen et al.[62]
Nucleoside derivatives	Inosine	CRC, bladder cancer, melanoma.	Mice	Untargeted metabolomics (serum and bacterial supernatant)	*Bifidobacterium pseudolongum*	Mice (in vivo; in vitro)	Adaptive (CD8 and CD4 T cells)	Immunostimulatory	Gut	Anti PD-L1/ CTLA-4	Mager et al.[55]
	Inosine	Melanoma and breast	Mice and human	Untargeted metabolomics (plasma)		Mice (in vivo; in vitro)	Tumour cells	Immunostimulatory	Gut	Anti PD-1/ CTLA-4	Zhang et al.[63]

https://doi.org/10.1038/s41467-026-72178-1

Table 2 (continued) | Overview of metabolites influencing anti-tumour immunity and ICB response

Metabolite type	Metabolite	Cancer type	Model (discovery)	Method used	Bacteria involved	Model (validation)	Immune compartment/ tumour	Immune effect	Gut vs tumour microbiome	Treatment type	Study
Polyunsaturated fatty acids	DHA	Melanoma	Mice	Untargeted metabolomics (stool)	*Lachnospirascease*	Mice (in vivo); human (in patient validation of faecal and tumour DHA)	Tumour cells	Immunostimulatory	Gut	Anti PD-L1	Teng et al.[66]
Coenzymes and vitamins	Pantothenate and CoA and	Melanoma	Mice	Untargeted metabolomics (serum and CD8 Tc)		Mice (in vivo; in vitro); human (in patient validation of serum pantothenate; in vitro)	Adaptive (CD8 T cells)	Immunostimulatory	Unknown	Adoptive T cell therapy, anti PD-L1/PD-1	St. Paul et al.[60]
	Riboflavin and 5-OP-RU	MSI tumours (Pan-cancer)	Human	Untargeted metabolomics (stool)		Human (in vitro)	Innate-like (MAIT cells)	Immunosuppressive	Gut	Anti PD-1	Mimpen et al.[38]
TCA Cycle metabolite	Succinate	CRC	Human	Untargeted metabolomics (stool, serum, and bacterial supernatant)	*F. nucleatum*	Mice (in vivo); human (in vitro, clinical cohort follow-up)	Tumour cells	Immunosuppressive	Gut	Anti PD-1	Jiang et al.[69]
	Succinate	Multiple myeloma	Succinate	Targeted metabolomics (stool and serum)		Mice (in vivo); human (in vitro)	Adaptive (CAR-T cells)	Immunostimulatory	Gut	Adoptive T cell therapy	Uribe-Herranz et al.[61]
Other microbial-derived bioactive compounds	UA	CRC	Targeted selection (direct focus on UA)	No metabolomics		Mice (in vivo; in vitro); human (in vitro)	Adaptive (CD8 T cells)	Immunostimulatory	Gut	Adoptive T cell therapy	Denk et al.[59]
	Phytosphingosine	Breast cancer (TNBC, CRC)	Mice and humans (in vitro screen)	Untargeted metabolomics (Postbiotic mixture)	*Lacticaseibacillus paracasei* and *Akkermansia muciniphila*	Mice (in vivo; in vitro); human (in vitro)	Tumour cells	Immunostimulatory	Gut	Anti PD-1/ Adoptive T cell therapy	Ferrari et al.[64]
	Trigonelline	Bladder cancer	Mixed: human (bacteria) and mice (metabolite)	Untargeted metabolomics (serum)	*Blautia coccoides*	Mice (in vivo)	Tumour cells	Immunostimulatory	Gut	Anti PD-1	Wang et al.[67]
	HMBPP	Melanoma, NSCLC, RCC, MSI-cancers (pan-cancer)	Human	Metagenomics (stool)		Human (in vitro)	Innate-like (Vγ9 Vδ2 T cells)	Immunostimulatory	Gut	Anti PD-1	Mimpen et al.[38]

MSS-CRC microsatellite stable colorectal cancer, *MSI* microsatellite instable, *NSCLC* non-small cell lung cancer, *PDAC* pancreatic ductal adenocarcinoma, *TNBC* triple-negative breast cancer, *RCC* renal cell carcinoma, *HCC* hepatocellular carcinoma, *TKI* tyrosine kinase inhibitor.

https://doi.org/10.1038/s41467-026-72178-1

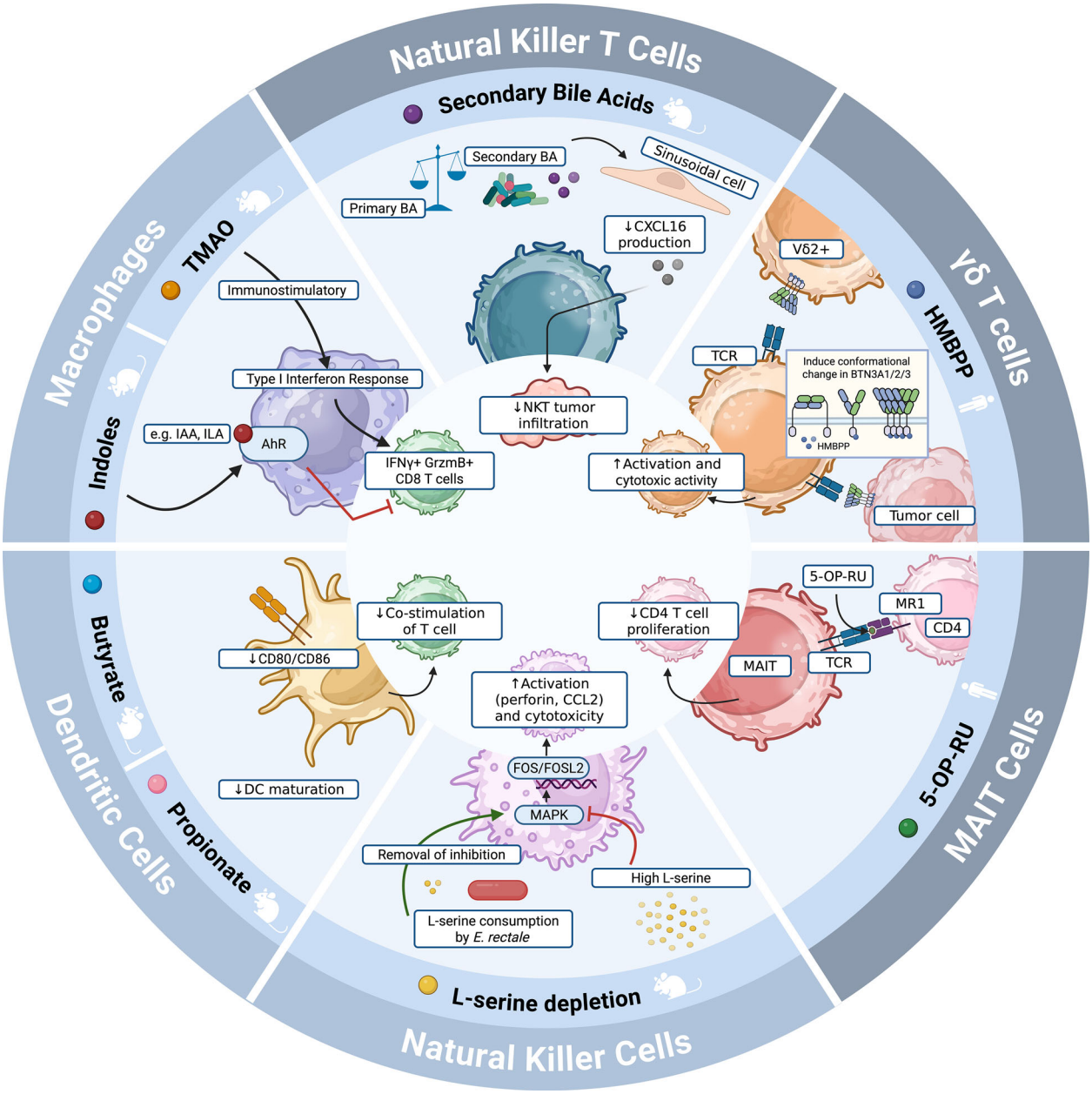


Fig. 1 | Microbial metabolites shape innate and innate-like anti-tumour immunity. Microbiota-derived metabolites modulate both the innate-like (NKT cells, γδ T cells and MAIT cells [dark blue]) and innate (macrophages, DCs, and NK cells [light blue]) immune system, thereby impacting anti-tumour immunity. Mechanistically, secondary bile acids inhibit CXCL16 production by liver sinusoidal endothelial cells, which hinders NKT cell trafficking to the tumour. HMBPP binds to and induces a conformational change in BTN3A1/2/3 on both tumour and γδ T cells, leading to Vδ2 γδ T cells activation through interaction with the TCR. Presentation of 5-OP-RU on MR1 on CD4 T cells binds to the MAIT TCR, thereby activating MAIT cells, which in turn inhibit CD4 T cell proliferation. Depletion of L-serine by *E. rectale* promotes activation of NK cells via the MAPK pathway. The SCFAs butyrate and propionate reduce response to anti-CTLA-4 through downregulation of CD80/86 on DCs, which prevents co-stimulation of T cells. TMAO induces a type I interferon-dependent immunostimulatory phenotype in macrophages, which leads to CD8 T cell activation. Indoles, including IAA, bind to the AhR on macrophages to induce an immunosuppressive phenotype. Mouse studies are depicted by a mouse icon, while the association of the metabolite with immunotherapy outcome in human clinical studies is depicted by a human icon. BA, bile acid. NKT, natural killer T cell. HMBPP, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate. TCR, T cell receptor. BTN3A, butyrophilin subfamily 3 member A. 5-OP-RU, 5-(2-oxopropyli-deneamino)-6-d-ribitylaminouracil. MR1, major histocompatibility complex class I-related protein. MAIT, mucosal-associated invariant T cell. *E. rectale*, *Eubacterium rectale*. MAPK mitogen-activated protein kinase, DC dendritic cell, AhR aryl hydrocarbon receptor, GrzmB granzyme B, TMAO trimethylamine-N-oxide. Created in BioRender. Voest, E. (2026) https://BioRender.com/jpq4s68.

tumour. Finally, in patients with pancreatic cancer, lower levels of AHR expression were associated with improved overall survival

Although understudied, DCs can also be modulated by microbiota-derived metabolites. Initial evidence came from a study by Nastasi et al., which showed that treating healthy-donor-derived DCs with the SCFAs (diet-microbiota, Box 1) butyrate and propionate in vitro reduced the production of pro-inflammatory cytokines and induced an anti-inflammatory profile in these cells[23]. Later, this was confirmed by Coutzac et al., who demonstrated that higher serum levels of butyrate and propionate were associated with poor response to anti-CTLA-4 therapy in patients with metastatic melanoma[24]. In a mouse model of three distinct cancer types, the authors demonstrated

that anti-CTLA−4-mediated DC maturation was decreased upon the addition of butyrate in the drinking water, as evidenced by lower CD80/CD86 expression on these cells. This resulted in reduced co-stimulatory CD28 signalling, which led to a diminished adaptive immune response, hampering the efficacy of anti-CTLA-4.

Despite few studies directly linking microbiota-derived metabolites to NK cell activity in relation to immunotherapy response, direct effects of microbial metabolites on NK cells were reported. An example of this is the SCFA butyrate, which was shown to increase proliferation of a human immortalised NK cell line, enhance its anti-tumour cytotoxic capacity, and reduce anti-inflammatory IL-10 production[25]. This is in contrast with the previously described immunosuppressive effect of butyrate on DCs. However, these effects of butyrate on NK cell activity were only tested in vitro, in the absence of a tumour, which may explain the observed discrepancies. Additionally, Liu et al. demonstrated in a tumour-bearing mouse model that *Eubacterium rectale*, which is enriched in patients with melanoma responding to anti-PD1, can enhance anti-PD1 efficacy by improving NK cell function and infiltration[26]. In these tumour-bearing mice, *E. rectale* was shown to consume and hence deplete L-serine, as reflected by lower serum and faeces concentrations. This decrease in L-serine, which normally suppresses the mitogen-activated protein kinase (MAPK) pathway, led to activation of FOS/FOSL2 signalling in NK cells, thereby boosting their activity and improving anti-PD1 efficacy in mice. Furthermore, key enzymes in L-serine metabolism were also negatively associated with CD4, CD8 and γδ T cell infiltration in the tumour of anti-PD1-treated patients with melanoma, which further suggests a link between L-serine and anti-tumour immunity. However, other studies have shown both in vitro and in vivo that the impact of L-serine depletion on cancer cell proliferation is highly dependent on the genetic landscape of the tumour[27,28], highlighting a context-dependency that may similarly shape its effect on the immune system. Additionally, given that L-serine is a non-essential amino acid and usually readily available through dietary intake, further studies in human patients will be essential to confirm the translational relevance of these observations.

Altogether, these studies demonstrate the ability of the microbiome to influence innate immune cell activity, thereby shaping anti-tumour immunity and modulating response to ICB (Fig. 1). However, despite the central role of innate immune system in sensing microbes, only few microbiota-derived metabolites, including the widely studied SCFAs and tryptophan derivatives, have been shown to affect these cells in the context of immunotherapy. Moreover, the contribution of other innate immune cells, such as neutrophils and eosinophils, to metabolite-immune crosstalk remains largely unexplored, despite their established roles in anti-tumour immunity[29–31]. Advancing this field will require large clinical data sets linking microbial functionality to ICB response to select candidate metabolites, followed by functional assays to validate their immunomodulatory effect. Finally, these studies show that metabolites, such as butyrate, can exert both immunostimulatory and immunosuppressive effects, depending on the tumour or immune cell type examined and the model system used, further highlighting the complexity of metabolite-mediated immune regulation.

Innate-like immunity

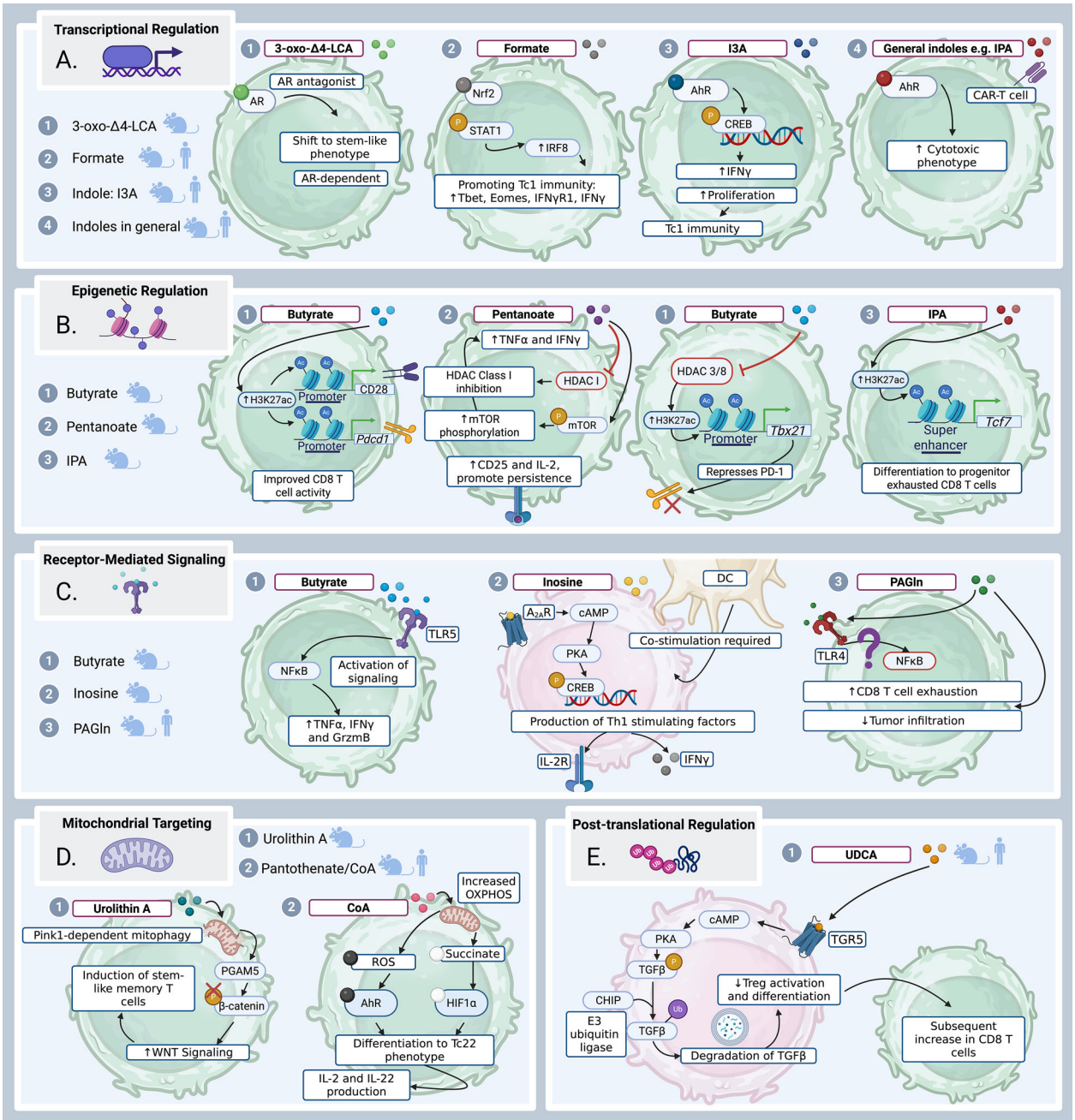
Innate-like immune cells are bridging the innate and adaptive immune system and include cells such as innate lymphoid cells (ILCs) and innate-like T cells. Some of these innate-like T cells, including mucosal-associated invariant T (MAIT) cells, γδ T cells, and NKT cells, are known to recognise bacterial-derived products, thereby playing an important role in pathogenic defence[32–34]. Moreover, these innate-like T cells have emerged as critical players in anti-tumour immunity, as recently reviewed by Ruf et al. [35]. For example, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP; de novo production, Box 1), an intermediate in the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway in bacteria, is a potent activator of Vγ9Vδ2T cells[36], which is a subset of γδ T cells capable of direct tumour cell killing and presentation of tumour antigens to CD8 T cells[37]. Indeed, we recently showed a significant association between microbial MEP pathway abundance in the gut through faecal metagenomics and response to ICB in patients with different types of cancer[38]. Supernatant of bacteria capable of producing HMBPP promoted Vδ2 T cell-mediated killing of patient-derived tumour organoids. Moreover, another subset of innate-like T cells, MAIT cells, can recognise and be strongly activated by bacterial metabolites derived from riboflavin synthesis, such as 5-OP-RU (de novo production, Box 1), when presented by the major histocompatibility complex-related 1 (MR1) molecule[39,40]. In the context of immunotherapy, we recently found that patients with mismatch repair-deficient (MMRd) cancers who were resistant to ICB showed an enrichment of riboflavin synthesis pathways in their gut microbiome, as determined by metagenomics, along with increased levels of MAIT cells in the tumour[38]. This detrimental effect of riboflavin on ICB response was further confirmed by metabolomics on faecal samples of these patients, which showed that high riboflavin levels were linked to worse survival in patients with high levels of MAIT cells in the tumour. Furthermore, Li et al. demonstrated an association between the activation of MAIT cells and tumour-infiltrating bacteria in human colorectal cancer (CRC)[41]. In vitro treatment of MAIT cells with both live bacteria and supernatant from the CRC-associated bacterium *Fusobacterium nucleatum* potently activated MAIT cells via the MR1 molecule, suggesting that the mechanism of action is mediated by a secreted factor. However, the specific metabolite exerting this effect was not identified[41]. Finally, NKT cells were found to protect against tumour growth in the liver in both primary hepatocellular carcinoma and liver metastasis mouse models, which was, however, impaired by a defective conversion of primary bile acids to secondary bile acids (host-microbiota, Box 1) by intestinal *Clostridium* species. Whereas primary bile acids in the liver can increase CXCL16 expression by liver sinusoidal endothelial cells, thereby inducing NKT cells recruitment into the tumour and enhancing anti-tumour immunity, secondary bile acids exert the opposite effect. Therefore, in mice with a high abundance of bacteria mediating primary-to-secondary bile acid conversion, elevated levels of secondary bile acids in the liver prevented recruitment of NKT cells into the liver and thus promoted tumour growth[42].

Despite the known role of innate-like immune cells in sensing microbiota-derived metabolites, few studies have explored the relevance of these cells in the context of anti-tumour immunity (Fig. 1). This lack of studies focusing on the innate-like immune compartment may be partially explained by the many studies using mouse models to both identify and assess microbiota-derived metabolites relevant to immunotherapy (Table 2). However, it is important to consider that, in general, mouse models do not adequately reflect these innate-like immune subsets such as γδ T cells[43,44] and MAIT cells[45]. Therefore, integrated patient datasets and human model systems are needed to provide insights into the complex interactions between metabolites and innate-like immune cells.

Adaptive immunity

Even though innate immune cells form the first line of defence and are the initial sensors of microbes, several microbiota-derived metabolites have also been shown to directly affect adaptive immune cells. Unsurprisingly, a large proportion of studies investigating the role of microbiota-derived metabolites in immunotherapy success have focused on the adaptive immune system, particularly CD8 T cells, as these cells are crucial for response to ICB. Across studies, microbiota-derived metabolites exert their effects on the adaptive immune system through 5 different mechanisms: (1) transcriptional regulation, (2) epigenetic regulation, (3) receptor-mediated signalling activation, (4) regulation of mitochondrial function, and (5) post-transcriptional

https://doi.org/10.1038/s41467-026-72178-1



regulation. These mechanisms are summarised in the following paragraphs and Fig. 2.

Transcriptional regulation

A number of metabolites, including formate[46], indole-3-aldehyde (I3A)[47], IPA[48], and bile acids[49] enhance anti-tumour immunity through regulating the transcription of immune-related genes. In a melanoma mouse model, Phelps et al. discovered in serum and caecal samples an upregulation of formate production by the microbiome upon exercise[46]. Formate can be produced by the microbiome as a by-product of anaerobic fermentation, one-carbon metabolism and pyruvate metabolism (diet-microbiota, Box 1). Further investigation into formate revealed that its administration stimulated anti-tumour immunity and restricted tumour growth in melanoma, lymphoma, and CRC mouse models. This effect was further enhanced in combination with anti-PD-L1. Mechanistically, formate activates the transcription factor nuclear factor erythroid 2-related factor (Nrf2) in CD8 T cells, thereby promoting a cytotoxic Tc1-type of immune response. Additionally, serum levels of formate were higher in patients with melanoma responsive to anti-PD1 and were associated with improved progression-free survival. Another transcription factor influenced by a microbiota-derived metabolite is the AhR, which binds I3A[47]. The impact of this metabolite on immunotherapy response was demonstrated by the fact that the probiotic *L. reuteri* could promote effector T cell responses in a melanoma mouse model through production of I3A in the tumour microenvironment (TME). Binding of I3A to AhR in CD8 T cells induces phosphorylation of cAMP response element-binding protein (CREB), resulting in increased proliferation, as well as IFN-γ and granzyme B production in these cells. This occurred specifically within the TME, as *L. reuteri* present at other sites was not sufficient to reduce tumour growth. Interestingly, increased levels of I3A in the serum of patients with melanoma are associated with a favourable response to ICB. In line with this AhR-mediated CD8 T cell activation, the presence of *Akkermansia* spp. in the gut enhanced CAR T cell efficacy against B cell lymphoma in mice through the production of AhR-binding indoles. These indoles, including IPA, then entered the

Fig. 2 | Microbiota-derived metabolites regulate adaptive immune activity. Microbiota-derived metabolites modulate the adaptive immune system and affect ICB response via different mechanisms. **A** The metabolites 3-oxo-Δ4,6-LCA, formate, and I3A modulate immunotherapy response at the level of transcriptional regulation. 3-oxo-Δ4,6-LCA binds to AR, thereby inducing a stem-like phenotype in CD8 T cells (green). Formate binds to Nrf2, leading to phosphorylation of STAT1 and increased expression of IRF8, thereby promoting cytotoxic Tc1 immunity. Tc1 immunity is further promoted by I3A binding of AhR, leading to phosphorylation of CREB and changes in gene expression. The binding of indoles, e.g. IPA to AhR, also boosts the cytotoxic capacity of CAR-T cells. **B** Butyrate, pentanoate and IPA modulate immunotherapy response through epigenetic changes. Butyrate increases H3K27ac of the promoters of CD28 and PD1. Pentanoate inhibits class I HDACs and induces phosphorylation of mTOR to increase TNFα and IFNγ production by CD8 T cells and improve their persistence. Additionally, butyrate also inhibits HDAC 3/8 in CD8 T cells to upregulate *Tbx21*, a negative regulator of PD1. IPA induces H3K27ac of the super-enhancer of *Tcf7*, thereby inducing differentiation of CD8 T cells to progenitor exhausted cells. **C** Receptor-mediated signalling via butyrate, inosine and PAGln modulates anti-tumour immunity. The binding of butyrate to TLR5 activates the NFκB signalling pathway, which increases the effector potential of CD8 T cells. Inosine, through binding of $A_{2A}R$, activates the cAMP-PKA-pCREB pathway, thereby promoting CD4 (pink) Th1 function. PAGln promotes CD8 T cell exhaustion and decreases infiltration of T cells into the tumour. **D** Mitochondrial targeting through UA or pantothenate/CoA leads to enhanced T cell function. UA induces mitophagy, thereby releasing PGAM5, which modulates signalling pathways to induce stem-like memory T cells. Pantothenate or CoA increase mitochondrial OXPHOS, leading to ROS and succinate production, ultimately resulting in Tc22 differentiation, a cytotoxic effector T cell subset. **E** UDCA, through binding of bile acid receptor TGR5, induced degradation of TGFβ. Treg activation and differentiation were therefore diminished, allowing improved CD8 T cell function. In vivo validation is depicted by a mouse icon, while the association of the metabolite with immunotherapy outcome in human clinical studies is depicted by a human icon. I3A indole-3-aldehyde, AR androgen receptor, Nrf2 nuclear factor erythroid 2-related factor 2, STAT1 signal transducer and activator of transcription 1, IRF8 interferon regulatory factor 8, AhR aryl hydrocarbon receptor, CREB cAMP response element-binding protein, IPA indole-3-propionic acid, HDAC histone deacetylase, mTOR mammalian target of rapamycin, PD1 programmed cell death protein 1, TLR4/5 toll-like receptor 4/5, $A_{2A}R$ adenosine A2A receptor, cAMP cyclic adenosine monophosphate, PKA protein kinase A, PAGln phenylacetylglutamine, PGAM5 phosphoglycerate mutase family member 5, CoA coenzyme A, OXPHOS oxidative phosphorylation, ROS reactive oxygen species, HIF1a hypoxia-inducible factor 1-alpha, UDCA ursodeoxycholic acid, CHIP carboxyl terminus of Hsc70-interacting protein, TGR5 Takeda G protein-coupled receptor 5. Created in BioRender. Voest, E. (2026) https://BioRender.com/gjw9wr8.

system and bound AhR on CAR T cells, thereby promoting their homing to the bone marrow and improving its effector function. Consistently, in patients with B-cell lymphoma receiving CAR T cells, the presence of *Akkermansia* in the stool correlated with higher levels of indoles in the plasma[48]. In a separate study, Jin et al. combined computational and experimental screening to functionally profile the poorly characterised microbiota-derived bile acids in human and mouse faeces. This led to the identification of microbiota-derived bile acids (host-microbiota, Box 1) that can modulate anti-tumour immunity through antagonising the transcription factor androgen receptor (AR) in CD8 T cells[49]. Specifically, the bile acid 3-oxo-Δ4-LCA improves anti-PD1 response in a bladder cancer mouse model by inducing an AR-dependent shift in CD8 T cells to a stem-like phenotype. Additionally, the presence of these specific bile acids was confirmed in both human serum and faecal samples. In contrast, Varanasi et al. demonstrated a role for the secondary bile acids LCA and isoalloLCA in hampering the function of mouse and human CD8 T cells in vitro[50]. However, this was through a different mechanism relating to increased exhaustion and an endoplasmic reticulum (ER) stress response. Expanding upon this, in a mouse model of liver cancer, LCA-fed mice showed increased tumour burden and lower CD4 and CD8 T cell infiltration. Collectively, these studies show that microbiota-derived metabolites directly interact with transcription factors to regulate gene expression of immune cells, thereby modulating their immune activity.

Epigenetic regulation

Several microbiota-derived metabolites, including butyrate[51–53], pentanoate[51], and IPA[54] are capable of epigenetically altering immune function through the modification of histone proteins or chromatin structures. For example, two independent studies have shown that butyrate improves response to adoptive cell therapy[51] and anti-PD1[52] through the inhibition of histone deacetylases (HDACs). Specifically, Luu et al. demonstrated that *Megasphaera massiliensis*-derived SCFAs enhanced cytotoxic lymphocyte effector functions. Treatment of human and murine CAR T cells with the SCFAs pentanoate and butyrate inhibited class I HDACs and increased phosphorylation of mammalian target of rapamycin (mTOR) in these CD8 T cells, thereby improving their effector function both in vitro and in pancreatic cancer-bearing mice. Additionally, pentanoate increased the expression of CD25 and IL-2 in CD8 T cells, enhancing their in vivo persistence via autocrine signalling[51]. Similarly, Wang et al. showed that intratumoral *F. nucleatum*-derived butyrate suppressed HDAC 3/8 in CD8 T cells in a mouse model of microsatellite stable (MSS) CRC, thereby alleviating their exhaustion and improving their effector function[52]. This butyrate-mediated inhibition of HDAC 3/8 led to an increase in H3K27 acetylation (H3K27ac) of the *Tbx21* promoter, which is a repressor of PD1 expression. As a result, CD8 T cells showed reduced PD1 expression and improved effector functions. Another study examining butyrate[53], similarly identified modulation of H3K27ac as a mechanism of modulating anti-tumour immunity by this metabolite. Here, butyrate upregulated PD1 on healthy-donor-derived CD8 and Vδ2 T cells by inducing increased H3K27ac at the promoters of *Pdcd1* and *CD28*. This increased expression of PD1 and CD28 on CD8 T cells resulted in enhanced CD8 T cell activity, and treatment with butyrate improved anti-PD1 responses in melanoma-bearing mice[53]. This butyrate-mediated upregulation of PD1 on CD8 T cells contrasts with the results of Wang et al., who showed that butyrate reduced PD1 expression on these cells. These conflicting results may potentially be explained by differences in model systems used (CRC-bearing mice vs human healthy-donor-derived CD8 T cells) and the dosing and concentration of butyrate (bacterial supernatant vs purified metabolite). Nonetheless, both studies report improved effector function of CD8 T cells upon treatment with butyrate, as demonstrated by the increased production of IFN-γ and TNF-α in vivo. It should be noted, however, that butyrate functions as a broad HDAC inhibitor and can act on multiple cell types, potentially eliciting divergent effects. Finally, cross-feeding between *L. johnsonii* and *C. sporogenes* (Box 1) in the gut increased serum levels of IPA, which promoted H3K27ac at the super-enhancer region of *Tcf7*, thereby driving CD8 T cell differentiation towards a progenitor exhausted state. This was associated with improved efficacy of anti-PD1 treatment in mice bearing MMRd CRC[54].

Receptor-mediated signalling activation

Microbial metabolites can influence distinct immune signalling pathways essential for coordinating immune function. Molecules like, but not limited to, inosine[55], butyrate[56], and phenylacetylglutamine (PAGln)[57] have established immune signalling-modulating functions. For example, Mager et al. showed in a mouse model of CRC that intestinal *Bifidobacterium pseudolongum* elevated serum levels of inosine, a metabolite which can be produced by bacteria predominantly through the purine salvage pathway. Inosine, in turn, promoted Th1 differentiation through binding to the adenosine 2A receptor ($A_{2A}R$) on T cells, which leads to activation of the $A_{2A}R$-cAMP-

PKA-CREB pathway. This activation subsequently results in the production of Th1-stimulating factors, including IL-12 receptor and IFN-γ, thereby boosting ICB response[55]. This beneficial effect of inosine on anti-CTLA-4 response in mice requires co-stimulation by DCs, as in the absence of DCs, increased tumour size and decreased CD4 and CD8 T cell infiltration were observed. Additionally, Kang et al. showed that *Roseburia intestinalis*-derived butyrate, as detected by metabolomics on bacterial conditioned media and mice faeces, enhanced the response to anti-PD1 treatment in a mouse model of MSS-CRC. Butyrate bound the toll-like receptor 5 (TLR5), which resulted in NF-κB activation and enhanced CD8 T cell function[56]. Additionally, through performing a multi-omics analysis of the gut microbiome and metabolome in a pan-cancer cohort of patients treated with ICB, Zhu et al. found PAGln (microbiota-host, Box 1) to be negatively correlated with anti-PD1/PD-L1 efficacy. This metabolite promotes CD8 T cell exhaustion and hampers their infiltration into the tumour in a CRC mouse model[57]. Interestingly, PAGln was previously shown to inhibit T cell activity in vitro in mouse spleen cells by suppressing NF-kB and TLR4 signalling[58]. Taken together, although these metabolites target different signalling pathways, they collectively demonstrate the interaction of metabolites with T cell surface receptors. This interaction results in the downstream activation or inhibition of key signalling pathways and therefore influences immune activation and responsiveness to ICB.

Regulation of mitochondrial function

Emerging evidence has highlighted the capacity of microbiota-derived metabolites to modulate mitochondria in T cells, which can lead to altered immune metabolism and function. For example, administration of the metabolite UA (diet-microbiota, Box 1), which can be produced by gut commensals, suppresses tumour growth and enhances adoptive cell therapy in CRC-bearing mice through inducing Pink1-dependent mitophagy in CD8 T cells. Mitophagy is the specific degradation and removal of mitochondria, which in this case led to the release of the mitochondrial protein phosphatase phosphoglycerate mutase family member 5 (PGAM5) and dephosphorylation of β-catenin. This resulted in increased Wnt signalling and the induction of stem-like memory T cells (T_{SCM}) in vitro in both human and murine T cells exposed to UA[59]. These T_{SCM} cells can self-renew and therefore overcome the exhausted T cell state commonly observed in the TME, thereby improving the adoptive cell therapy outcome. Additionally, St. Paul et al. showed that co-administration of pantothenate (vitamin B5; de novo production, Box 1) with anti-PD-L1 treatment reduced tumour growth in a mouse model of CRC[60]. Mechanistically, the authors showed in vitro that coenzyme A (CoA), a breakdown product of pantothenate, can trigger increased oxidative phosphorylation in T cells. This led to reactive oxygen species (ROS)-induced activation of AhR and succinate-induced hypoxia-inducible factor 1-α (HIF-1α) stabilisation in CD8 T cells. These changes in T cell metabolism ultimately stimulate the differentiation of CD8 T cells to a Tc22 phenotype capable of producing IL-2 and IL-22, which was shown to boost anti-tumour immunity in vivo. Moreover, although demonstrated in a small patient cohort, elevated stool levels of succinate were associated with enhanced CAR T cell effector functions and improved persistence in patients with multiple myeloma. In vitro, exposure of CAR T cells to succinate increased their respiratory capacity, consistent with a more memory-like T cell profile, which may underlie the observed improvement in persistence[61]. Altogether, these studies highlight the direct impact of microbiota-derived metabolites on mitochondrial function and their downstream effect on immune cell function and anti-tumour immunity. However, as mitochondria are complex organelles involved in many cellular processes, including metabolism, apoptosis, and signalling, it remains challenging to pinpoint the direct regulatory effects of microbiota-derived metabolites.

Post-translational regulation

Shen et al. demonstrated that administration of UDCA (host-microbiota, Box 1), a secondary bile acid produced by intestinal bacteria, leads to the degradation of TGF-β through post-translational modification, thereby enhancing antitumor immunity in tumour-bearing mice[62]. UDCA binds to the bile acid receptor TGR5 on the membrane of CD4 T cells, which leads to increased levels of cyclic AMP (cAMP). This cAMP can, in turn, activate protein kinase A (PKA), leading to phosphorylation of TGF-β, which promotes its binding to and ubiquitination by carboxyl terminus of the Hsc70-interacting protein (CHIP). This ubiquitin-induced degradation of TGF-β by UDCA results in diminished regulatory T cell (Treg) activation and differentiation and therefore boosts response to anti-PD1/PD-L1 in CRC, melanoma, and lung cancer mouse models. Although this study shows the relevance of metabolite-induced post-translational modification in immune cells, this area remains largely underexplored. In the field of immune-oncology, most studies to date have investigated transcriptional regulation in immune cells and tumour cells, rather than post-transcriptional changes. Nevertheless, techniques to identify these post-translational modifications, including mass-spectrometry, may help to uncover novel pathways by which microbiota-derived metabolites influence anti-tumour immunity.

Taken together, these studies highlight the multifaceted effects of microbial metabolites on the adaptive immune system. Such effects may arise through distinct mechanisms, or through a combination of processes such as receptor-mediated signalling followed by post-translational regulation, as is the case for UDCA[62]. Notably, it is clear from the studies described that research has predominantly focused on CD8 T cells, highlighting a gap in knowledge for how these metabolites influence the function of CD4 T cells and B cells, which are also important players in the anti-tumour immune response.

Direct effects on tumour cells

Beyond the impact of microbiome-derived metabolites on immune cells, direct effects on tumour cells can also occur, which in turn lead to changes in the immune context of the tumour. These metabolites exert their effects mainly through two mechanisms: (1) modulation of tumour immunogenicity via MHC molecules and immune checkpoints, and (2) regulation of immune cell recruitment (Fig. 3).

Modulation of tumour immunogenicity

Several metabolites, including inosine[63], phytosphingosine[64], L-arginine[65], docosahexaenoic acid (DHA)[66], and trigonelline[67], impact MHC- or immune checkpoint expression. Inosine and phytosphingosine improve antigen presentation through upregulation of MHC class I on tumour cells, promoting their detectability by the immune system. For example, Zhang et al. showed an enrichment of inosine in the serum of patients with renal cell carcinoma and in tumour-bearing mice that responded to anti-PD1 and anti-CTLA-4 treatment. In vitro, inosine directly inhibits ubiquitin-like modifier-activating enzyme 6 (UBA6), which increases the expression of MHC-I and other genes involved in antigen presentation, thereby enhancing immune recognition[63]. Although this study did not directly describe a role for the microbiome, inosine can be microbiome-derived and, in turn, modulate Th1 cells activity, as was demonstrated by Mager et al. [55]. Additionally, Ferrari et al. examined the effect of supernatant derived from *Lacticaseibacillus paracasei* and *Akkermansia muciniphila*, bacterial species known to be associated with ICB response, on HLA class I expression on human tumour cells in vitro[64]. Through profiling the supernatant by metabolomics, they demonstrate that phytosphingosine in the *L. paracasei* supernatant potently increased HLA class I expression. Phytosphingosine activates the MyD88-NF-κB pathway, thereby inducing the expression of NOD-like receptor family CARD domain containing 5 (NLRC5), which is an MHC class I transactivator. Combination of the bacterial supernatant with anti-PD1

https://doi.org/10.1038/s41467-026-72178-1

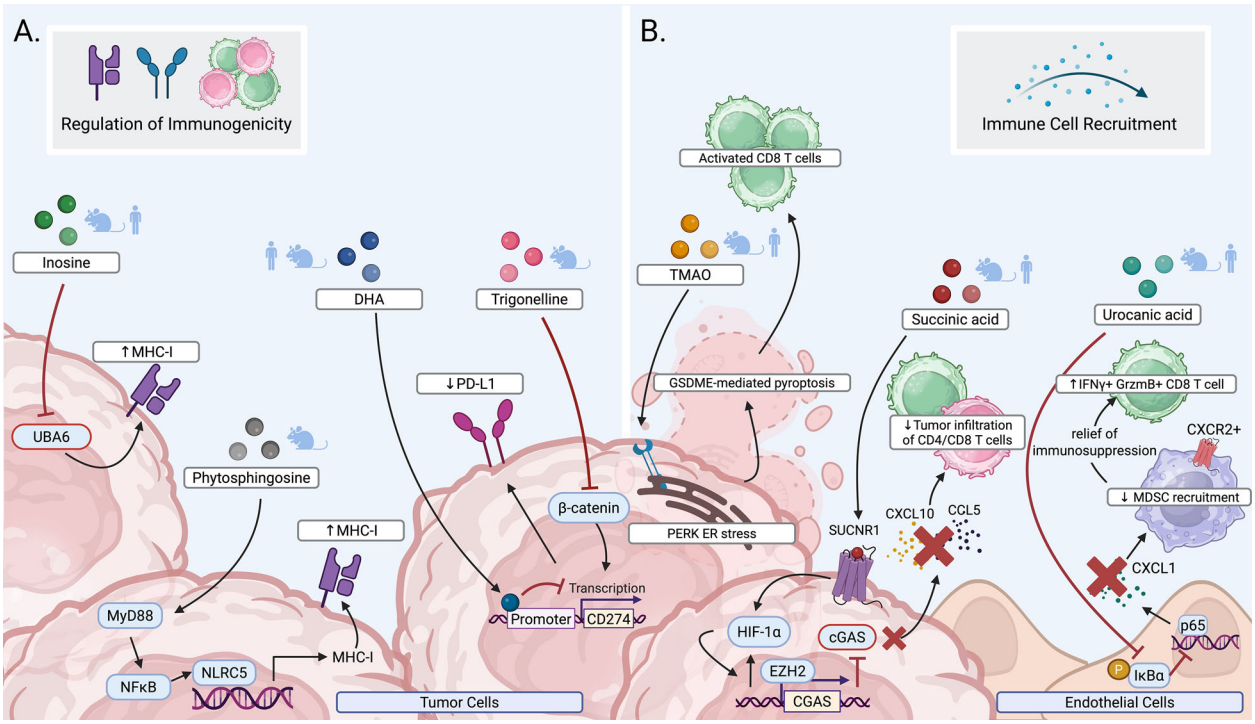


Fig. 3 | Direct effects of microbiota-derived metabolites on tumour immunogenicity. Recognition of tumour cells by the immune system can be impacted by microbiota-derived metabolites in two ways: **A** regulation of immunogenicity or **B** changes in immune cell recruitment. **A** Inosine and phytosphingosine achieve this through the upregulation of MHC-I on tumour cells. DHA and trigonelline block the transcription of PD-L1, thereby decreasing its expression and removing the brakes on T cells. **B** TMAO induces an immunogenic cell death in tumour cells via the induction of GSDME-mediated pyroptosis, the products of which activate CD8 T cells. Succinic acid binds to SUCNR1 on tumour cells, which decreases CXCL10 and CCL5 production, thereby hindering the infiltration of T cells into the tumour. UCA, through inhibition of IκBα on endothelial cells, lowers the concentration of CXCL1, resulting in reduced MDSC-mediated immunosuppression. In vivo validation is depicted by a mouse icon, while the association of the metabolite with immunotherapy outcome in human clinical studies is depicted by a human icon. UBA6 ubiquitin-like modifier-activating enzyme 6, MHC-I major histocompatibility complex I, MyD88 myeloid differentiation primary response gene 88, NLRC5 NLR family CARD domain containing 5, DHA docosahexaenoic acid, PD-L1 programmed death-ligand 1, TMAO trimethylamine-N-oxide, GSDME gasdermin E, PERK protein kinase R-like ER kinase, ER endoplasmic reticulum, SUCNR1 succinate receptor 1, EZH2 enhancer of zeste homologue 2, HIF-1a hypoxia-inducible factor 1-alpha, cGAS cyclic GMP-AMP synthase, MDSC myeloid-derived suppressor cell. Created in BioRender. Voest, E. (2026) https://BioRender.com/mp1uute.

therapy significantly reduced tumour growth compared to vehicle treatment in a mouse model of breast cancer and CRC. Moreover, Zhang et al. investigated the combination therapy of anti-PD-L1 with MEK inhibitors (MEKi) as they showed that MEKi increased MHC-I expression on tumour cells via NLRC5, both in vitro on human CRC cells and in CRC-bearing mice[65]. However, treatment with the MEKi also induced alterations in the gut microbiome of these mice, which diminished anti-PD-L1 efficacy. Specifically, the MEKi-induced shift in the microbiome resulted in elevated intestinal *Clostridiaceae*-derived arginase and thus decreased faecal, serum, and tumour levels of L-arginine, a metabolite essential for CD8 T cell function. Direct inhibition of arginase by the arginase inhibitor nor-NOHA boosted L-arginine concentration, resulting in enhanced CD8 T cell activation and ICB efficacy.

In addition to enhancing tumour immunogenicity via upregulating MHC expression, the metabolites DHA and trigonelline release tumour cell-induced immune suppression through downregulating PD-L1 expression on tumour cells. For example, administration of ginger-derived exosome-like nanoparticles (GELNs) improves response to anti-PD-L1 in melanoma mouse models through a *Lachnospirascease*-induced increase in the systemic concentration of DHA, which suppressed PD-L1 expression on tumour cells[66]. Mechanistically, the authors show in vitro that DHA binds the promoter region of PD-L1 in tumour cells, which blocks the recruitment of c-myc to this site, thereby inhibiting PD-L1 expression. Additionally, levels of intestinal DHA were elevated in patients with melanoma responsive to anti-PD-L1 treatment. Moreover, *Blautia coccoides*, which was found to be enriched in the gut microbiome of patients with bladder cancer, increases serum levels of trigonelline, which in turn enhances CD8 T cell antitumour immunity in a mouse model of bladder cancer. This occurs through its inhibition of β-catenin in tumour cells, leading to reduced tumour proliferation and decreased PD-L1 expression[67]. However, it should be noted that β-catenin is involved in numerous cellular processes, and therefore further validation in humans will be essential. Additionally, although trigonelline increased following the administration of *B. coccoides*, trigonelline is primarily a diet-derived metabolite, and the extent to which the microbiota contributes remains unclear.

Taken together, these studies highlight the potential role for microbiota-derived metabolites in boosting the immunogenicity of tumour cells. They also demonstrate the capacity of gut-derived metabolites to reach the tumour site and exert immunomodulatory effects. Based on this, exploration of combination therapies simultaneously targeting the gut and tumour may evolve as a promising strategy.

Regulation of immune cell recruitment and activation

Immune cell recruitment and activation are important factors for immunotherapy response. Tumour cells play an important role in the production of factors influencing immune cell recruitment and activation, including cytokines and chemokines. To date, several metabolites have been associated with tumour-mediated immune cell

recruitment and activation in relation to immunotherapy success. These include UCA[68], succinic acid[69], and TMAO[70]. UCA, a microbial metabolite derived from the breakdown of histidine, mediates the additive effect of tyrosine kinase inhibitors (TKIs) in combination with anti-PD1 treatment in CRC mouse models[68]. Performing untargeted metabolomics on plasma and faeces from both mice and patients treated with TKIs, the authors found an enrichment of UCA in the TKI-treated group. Mechanistically, UCA, produced by *Muribaculum*, directly acts on NF-κB inhibitor alpha (IκB) on tumour endothelial cells, resulting in a decrease in the production of CXCL1. This reduced level of CXCL1 leads to reduced myeloid-derived suppressor cell (MDSC) recruitment and increased CD8 T cell activation. Importantly, UCA did not show a direct effect on MDSCs or CD8 T cells, highlighting the importance of tumour endothelial cells. Interestingly, the abundance of *Muribaculum gordoncarteri* and UCA is associated with a favourable response to ICB in patients with CRC. Additionally, *F. nucleatum*, which associates with impaired immunotherapy efficacy in both patients with CRC, as well as CRC-bearing mice, modulated immune cell recruitment and impaired anti-tumour immunity through the production of succinic acid in a mouse model of CRC[69]. Metabolomic analyses of *F. nucleatum*-derived supernatant, as well as serum and faecal samples from two independent CRC patient cohorts, identified succinic acid as a candidate metabolite modifying response to ICB. Succinic acid is produced by both the host and the microbiota through the TCA cycle (diet-microbiota, Box 1). The authors show in vitro that succinic acid binds the succinate receptor 1 (SUCNR1) on human CRC cells, leading to stabilisation of HIF-1α and inducing increased expression of enhancer of zeste homologue 2 (EZH2), an enzyme involved in gene silencing. EZH2, in turn, binds the promoter of cyclic GMP-Amp synthase (cGAS), thereby silencing its expression. This downregulation of cGAS decreases the activation of stimulator of IFN response cGAMP interactor (STING), which reduces the production of CCL5 and CXCL10, factors that can induce migration of T cells to the tumour site, thereby decreasing anti-PD1 response. Furthermore, Wang et al. found a significant correlation between the level of the microbial metabolite TMAO in the tumour and an immune-activated TME in patients with triple-negative breast cancer (TNBC). Additionally, TMAO was able to induce CD8 T cell anti-tumour immunity and boost response to anti-PD1 in TNBC-bearing mice. In vitro, TMAO activated protein kinase R-like endoplasmic reticulum kinase (PERK) and induced ER stress, resulting in the induction of gasdermin E-mediated (GSDME) pyroptosis of murine tumour cells[70]. This pyroptosis is an immunogenic form of cell death that leads to inflammation through the release of cytokines such as IL-1β and IL-18[71], thereby promoting anti-tumour immunity.

In summary, these studies demonstrate that microbiota-derived metabolites can influence anti-tumour immunity, not only by directly modulating the immune system, but also by affecting tumour cells. Therefore, by focusing solely on immune cells, key metabolites that influence immunotherapy response may be easily overlooked.

Integrated approaches for assessing microbial functionality

Methodological considerations for profiling microbiome function

Microbiome-host interactions are a relatively new field, and faecal-derived metagenomics and metabolomics have emerged as powerful tools to investigate it. Although both approaches are crucial to gain an in-depth understanding of microbiome functionality in relation to immunotherapy response, they are not interchangeable. Whole-genome metagenomics is a robust strategy to functionally profile the microbiome by investigating the functions encoded by the annotated microbial genes. This method has several advantages, including the chemical stability of DNA and the availability of well-established protocols and reference databases. However, metagenomics can only outline the functional capacity of the microbiome as it does not indicate whether genes are indeed actively expressed[72]. In contrast, gut metabolomics provides a clear profile of the metabolites present, but is still in its infancy and therefore has several challenges[73]. For example, metabolomics is still lacking sufficient metabolite reference databases, leaving many metabolites without annotation in untargeted analyses. Additionally, metabolites are highly reactive, which can hinder their accurate measurement, with variability in methodologies and instruments further limiting data reproducibility. Furthermore, metabolites may have several functionally different isomers that cannot be distinguished by mass-spectrometry. Moreover, metabolites can be present at low, but functionally relevant concentrations, thereby not being detected by mass-spectrometry. Finally, many metabolites are not uniquely derived from the microbiome, complicating attribution of their effects to microbial activity alone, and raising the possibility that host metabolic pathways contribute indirectly. Considering these challenges, other omics approaches, such as metatranscriptomics and metaproteomics, could help identify which metabolic processes are active by revealing which microbial enzymes are being expressed, although these approaches are currently less commonly used[74]. These data would complement metagenomic and metabolomic data, and their integration would provide a broader view of the functional and metabolic state of the microbiome.

Importantly, it is crucial to consider the sampling site (plasma vs faeces) as these might provide different insights: whereas gut metabolomics directly reflects microbiome activity and local metabolite production, plasma metabolomics reflects systemic exposure. As a result, high faecal levels do not necessarily translate to systemic exposure, and plasma levels may reflect a mixture of microbial- and host-derived metabolism. This distinction is important because metabolites that act locally in the gut may not reach tumours or peripheral immune cells, while metabolites detected in the plasma are more directly linked to systemic immune modulation. Indeed, Sun et al. reported a poor correlation between faecal and plasma metabolomics in patients with CRC, further highlighting that these methods capture distinct aspects of host-microbiome interactions[75]. Finally, standardisation in sample collection, storage, experimental protocols, data analysis, and data deposition in open-access repositories, together with ongoing advances in technologies and reference databases, will further support the generation of reproducible microbial functional data. Several initiatives have been established to support this, including STORMS[76] (strengthening the organisation and reporting of microbiome studies) and the Human Microbiome Action[77], which provide structured frameworks aimed at harmonising the design, conduct, and reporting of microbiome research.

Integrative microbiome-host profiling

Combining microbiome-derived multi-omics data with approaches that profile host tumour- and immune compositions, including WGS, RNA-sequencing, and flow cytometry, will further advance the understanding of the impact of microbiota-derived metabolites on anti-tumour immunity. In addition, techniques that model spatial organisation of tumours, such as spatial transcriptomics, proteomics, and metabolomics, or an integrative combination thereof[78], may further help to elucidate interactions between the host, microbes, and their derived metabolites[79]. Through such integrative approaches, microbial metabolite levels can be correlated with the frequency and activation states of immune subsets, tumour characteristics, and clinical outcome. This may guide the identification of candidate metabolites, reveal the immune cell populations they influence, inform the selection of appropriate model systems for validations, and help disentangle local gut effects from systemic or tumour-specific effects, all within a clinically relevant patient setting. This integration can be achieved by collecting stool, plasma, and tumour samples from the same patients undergoing immunotherapy.

Candidate metabolite validation and clinical translation

To date, many studies have relied on (germ-free) mouse models (Table 2), both for the identification of metabolites of interest and for the assessment of their effect. While this has been important for showing causal effects of microbiota-derived metabolites on anti-tumour immunity, translation of these results to humans has not always been successful due to the differences in microbiome composition, immune subsets, and microbial metabolic capacity between (germ-free) mice and humans[80–82]. Additionally, differences in gastrointestinal tract anatomy, genetic backgrounds, as well as environmental factors, including diet, housing conditions, pathogen exposure, and exercise between mice and humans further complicates translation[83]. In the context of ICB, mice do not receive prior treatments, whereas patients, particularly in the metastatic setting, are often heavily pre-treated. Moreover, treatment regimens used in preclinical models frequently differ from real-world clinical practice, including differences between adjuvant, neo-adjuvant, and metastatic approaches, as well as between single-agent and combination therapies.

Results obtained in these studies must therefore be validated in models more representative of human biology, as well as in large clinical datasets. Several models would be suitable for this, including organoids[84,85], microfluidic devices (also known as organ-on-chip)[86,87], and the patient-derived tumour fragment (PDTF) platform[88]. These approaches allow the assessment of the interaction between human tumour and immune cells, either in a generated co-culture setting or their native context within an ex vivo tumour fragment. Treating these immune-tumour organoid co-cultures or PDTFs with bacterial supernatant or pure metabolites may help reveal or validate immunomodulatory effects in a human setting. For instance, bacteria producing the metabolite HMBPP were shown to potently induce the activity and anti-tumour cytotoxicity of Vδ2 T cells, an immune subset that is absent in mice[38]. Nevertheless, although these human models may complement findings in murine models, they also have inherent limitations, including their limited representation of the full complexity of the TME, the inability to disentangle local gut effects from systemic or tumour-intrinsic effects, and the restricted longevity of these cultures.

In addition to these differences between human and mouse studies, metabolites can have context-dependent effects that vary according to host metabolism and genetics, tumour-intrinsic biology, composition of the TME, (combinatorial) treatment strategies, metabolite dosing, and divergent effects on distinct cellular populations. One example of such context-dependency is the observation that microbial-derived riboflavin in the gut was associated with poor ICB response only in patients whose tumours contained high levels of MAIT cells, whereas this effect was not observed in patients with low levels of intratumoral MAIT cells[38]. These context-dependencies may therefore contribute to discrepancies between studies, and careful consideration of differences in tumour biology, immune cell type, model systems, and treatment regimens is crucial when interpreting results.

Together, the field will largely benefit from studies employing an integrated approach whereby large, longitudinal, multi-omics clinical data sets are leveraged to identify relevant microbial metabolites. Generation of these datasets will aid hypothesis generation, which requires validation in models representative of human anti-tumour immunity. Additionally, given the potential context-dependent effects of metabolites, findings obtained in a specific tumour type and with a specific ICB therapy cannot be directly translated to other settings and therefore require re-evaluation and validation.

Microbiota-based therapeutics for the enhancement of immunotherapy

Implementation of integrated research models, as well as functional validations will aid in the identification of metabolites which negatively or positively impact anti-tumour immunity. Accordingly, modulation of the levels of these specific metabolites and their downstream targets offers great potential to improve immunotherapy responses. Proposed approaches for altering microbiota-produced metabolite levels range from more broadly acting strategies, such as dietary modifications and FMT, to more specific strategies, including the administration of specific metabolites or bacteria producing them. An overview of these metabolite-targeting approaches is depicted in Fig. 4.

Interfering with the effect of microbial metabolites on anti-tumour immunity can be achieved using different strategies, each with its own advantages and limitations. These include: (1) modification of substrate availability, (2) administration of bacteria producing metabolites of interest, (3) removal of specific bacteria producing detrimental metabolites, and (4) direct interference with metabolite-receptor interactions or the downstream signalling pathway they activate. Firstly, metabolite production by bacteria is dependent on the availability of the relevant substrates. Approaches that aim to ensure availability of these substrates include dietary modifications or the administration of specific food-derivatives termed prebiotics. Studies focusing on dietary fibre consumption in ICB-treated melanoma patients identified an association between improved survival and higher fibre consumption[89,90]. However, these trials included low numbers of patients and were largely observational. Therefore, future studies would benefit from higher patient numbers and trial designs based on diet intervention, an approach being employed by the DIET trial[91], the results of which have not yet been published. Prebiotics such as ellagitannin[92], inulin[93], and mannose[94], have shown promise in murine studies, yet larger clinical trials are required to determine the effect in a human population. The benefits of these approaches include ease of application, low cost, and fewer administrative hurdles. However, clear limitations also exist, including the reliance on the pre-existing microbiome composition for substrate processing, as well as potential off target effects, whereby a single substrate can be the precursor for multiple metabolites.

Alternatively, bacteria or bacterial communities can be administered to shift the metabolic capacity of the microbiome. Such approaches include FMT, probiotics, bacterial consortia, and engineered bacteria. FMT has shown promise in improving ICB responses in clinical trials[10–12]. However, caution should be taken when interpreting these trial results due to low patient numbers, non-randomised trial designs and the possibility that restarting ICB may itself elicit a response[95]. Additionally, FMT has several disadvantages, including the lack of knowledge on the optimal composition for FMT, the risk of pathogen transfer, the lack of reproducibility, and the still poorly understood mechanism of action. The field is therefore shifting focus to the development of more defined bacterial products with distinct functions and metabolite production. The typical probiotic strategy involves the administration of live bacteria selected for their known associations with health, often with little mechanistic understanding[14,15]. Alternatively, bacterial consortia are a more optimised form of probiotics, consisting of groups of bacteria selected to grow well as a community and to synergistically perform a specific function. In this sense, bacterial consortia have notable benefits over the traditional probiotics: (1) they are designed based on a specified function, and (2) consortia species, due to in vitro training and community stability, have a greater likelihood of engraftment. While a promising approach, limited clinical data currently exist on the efficacy of bacterial consortia in the oncology field, and therefore the coming years will be critical for determining their therapeutic potential. Finally, in the interest of enhancing specific metabolite production, synthetically engineered bacteria can be employed. To date, the majority of studies that aimed to boost ICB efficacy have modified the non-pathogenic *Escherichia coli* strain Nissle 1917, for example, to convert waste products to L-arginine in the TME[96], or to produce IAA to

https://doi.org/10.1038/s41467-026-72178-1

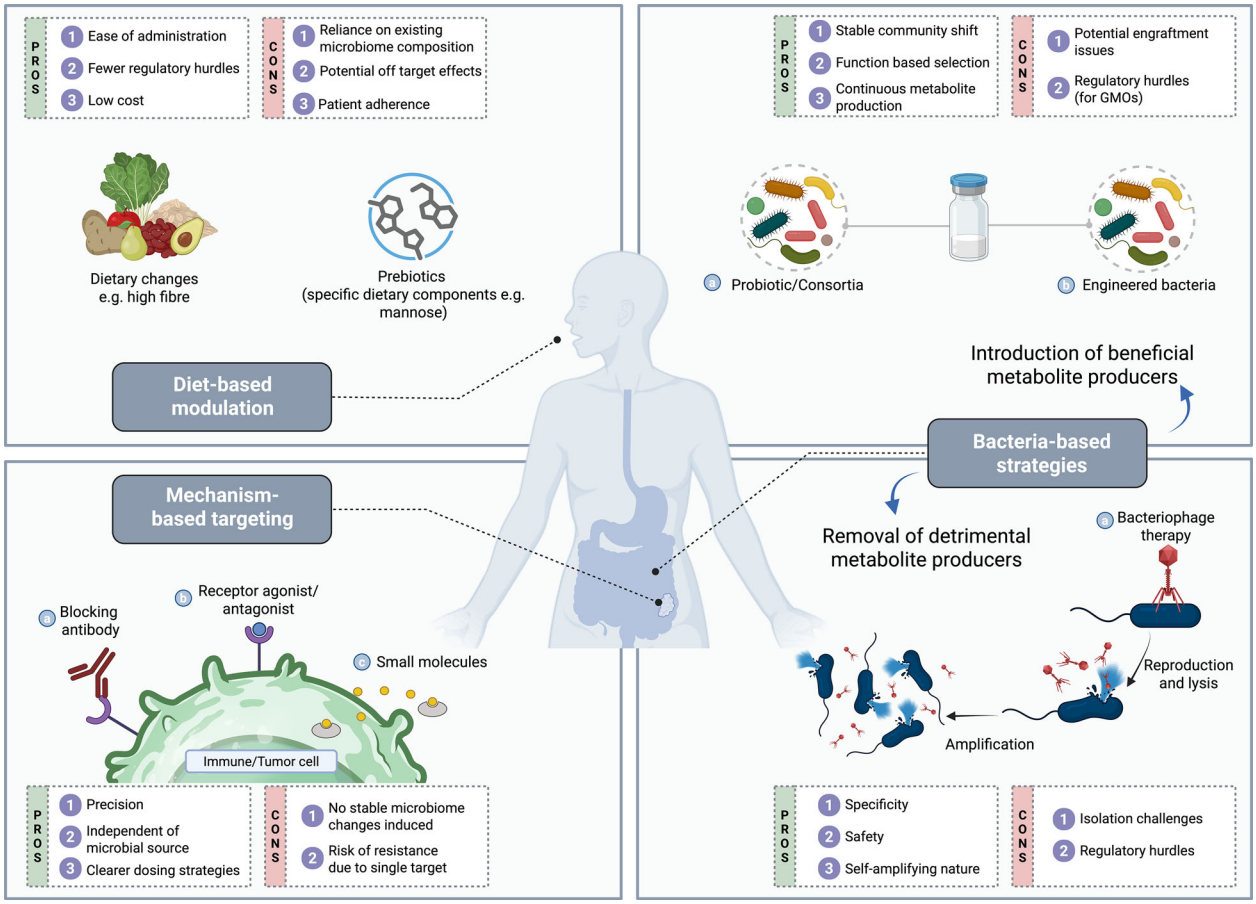


Fig. 4 | Modulating the microbiome to enhance immunotherapy efficacy. Several strategies can be applied to influence microbiota-derived metabolite production and its effects on immunotherapy. Three main approaches for this involve (1) diet-based modulation, which alters substrate availability, (2) bacteria-based strategies, which shift the metabolic capacity of the microbiome through the targeted introduction or removal of specific species and (3) mechanism-based targeting, which modulates known receptors or pathways involved in metabolite effects to achieve the desired outcome. Taken together, these strategies work to restore a disrupted microbiome and support anti-tumour immunity. Diet and microbial interventions adjust metabolite production, while mechanism-based targeting modulates the downstream effects of these metabolites. Created in BioRender. Toner, C. (2026) https://BioRender.com/6ip7g07.

promote T cell infiltration in the tumour[97]. Advantages of this approach include the stability and continuity of metabolite production. However, these theoretical advantages may also be considered high-risk disadvantages due to the long-lasting introduction of genetically modified organisms. Therefore, careful attention to the risks should be paid here.

A further distinct method involves the targeted removal of bacteria known to produce metabolites negatively impacting ICB response. For this, lytic bacteriophages (phages) can be employed to specifically lyse and kill a bacterial species of interest. This approach benefits from its specificity, self-amplifying nature, and proposed safety due to the narrow host range of phages. However, owing to advances in genomics analyses, recent studies have suggested that phages are not as specific as previously believed[98,99], indicating the need for further investigation into the possible consequences for phage therapy. Despite this, clinical safety has been shown in clinical trials for antimicrobial resistance[100,101], and their use to modulate the microbiome to enhance chemotherapy response[102,103] and anti-tumour immunity[104] has been shown to be effective in mouse models. Additionally, bacteriophage-based targeting has been demonstrated to have downstream effects on the metabolome[105]. Current limitations of this strategy relate to feasibility and clinical hurdles. Gut microbes warranting targeting are often anaerobic in nature and potentially lowly abundant in phage isolation sources, factors which have limited phage isolation for this purpose. Despite this, feasibility has improved in recent years with the development of methodologies suitable for bacteriophage isolation against anaerobic bacteria[106]. However, with the risk of resistance development, multiple phages targeting one species are required for phage therapy, requiring more high-throughput methods for isolation and testing. Moreover, regulatory approval for phages, as opposed to drugs, requires a unique approach due to their evolutionary capacity and host specificity. Altogether, phages provide an interesting avenue for exploration, however, key challenges should be addressed before this approach can be widely applied.

Finally, mechanistic studies will provide knowledge not only on key metabolites modulating immune cell function, but also on the receptors and pathways involved. Strategies that activate or block metabolite-bound receptors or interfere with the downstream pathways they induce open new doors for therapeutic development. Approaches for this include the development of antibodies, agonists and antagonists, or small molecules targeting these receptors and pathways. For example, a recent study investigated the use of the AB928/entrumadenant antagonist to block the adenosine $A2_A$ and $A2_B$ receptors on CAR-T cells, thereby relieving adenosine-induced immunosuppression[107]. This approach was found to be effective in a mouse model of colon carcinoma, yet further investigation is required to determine whether this is translatable to humans. Approaches such

as these offer advantages as they offer greater precision, do not rely on insight into microbial metabolite generation, and provide clearer dosing strategies.

Concluding remarks

There is overwhelming evidence that the microbiome has a direct influence on anti-tumour immunity, particularly through the production of metabolites. Many metabolites have been identified to exert immunomodulatory effects and thereby promote or hinder the efficacy of ICB. Metabolites can act on different subsets of the immune system or directly impact tumour cells. These novel insights have advanced our understanding of the complex interaction between the microbiome and the immune system and have paved the way for the development of microbiota-targeting interventions to combine with ICB.

Techniques to profile microbial functionality, including metagenomics and metabolomics, have significantly accelerated the identification of metabolites influencing ICB efficacy. However, major challenges still exist, and therefore, critical metabolites impacting anti-tumour immunity remain to be uncovered. Improving the sensitivity of mass-spectrometry, as well as enhancing reference databases for metabolite identification, will be a major step forward. Currently, integrating metagenomics and metabolomics with analyses of tumour-immune characteristics in ICB-treated patients offers a promising and unbiased approach to identify novel metabolites important for ICB response.

After identifying metabolites of interest, it is crucial to validate their immunomodulatory effects and decipher the mechanisms through which they influence anti-tumour immune responses. However, it needs to be considered that several of these microbiota-metabolites have been reported to induce contradictory immunomodulatory effects. For example, butyrate was reported to downregulate the expression of PD1 on CD8 + T cell in one study[52], whereas another study found that it induces PD1 expression[53]. Additionally, AhR engagement by indole metabolites in macrophages promoted an immunosuppressive phenotype, that hampered CD8 T cell activation[21], while AhR engagement on CAR T cells enhanced their effector function and improved persistence[48]. Finally, succinate was shown to bind to SUCNR1 on tumour cells and suppress factors that facilitate T cell trafficking into to the tumour[69], thereby reducing anti-PD1 efficacy, whereas in vitro succinate treatment of CAR T cells improved its effector function[61]. These contradictory effects may reflect differences in tumour types, ICB treatment strategies (e.g. single agent vs multi-agent, or adjuvant vs neoadjuvant approaches), and/or model systems (e.g. mice vs human). Moreover, metabolites can act on distinct cell populations and thereby exert divergent effects, or they may exert effects only under specific conditions, for example, depending on host genetics or the composition of the TME, highlighting that metabolite-mediated immune modulation can be highly context dependent. Together, this highlights the need to validate the effects of microbiome-derived metabolites in larger patient cohorts and in functional assays using carefully selected model systems before translating these molecules to the clinic. Given the substantial differences between mice and humans in both microbiome composition and immune function, integration of human datasets and model systems will be essential to facilitate this translation.

Finally, most studies on metabolite-mediated immune modulation have focused on the adaptive immune system, particularly CD8 T cells. However, the potential impact on other adaptive immune cell subsets, including CD4 T cells and B cells, warrants further investigation. Additionally, in recent years, the importance of the innate and innate-like immune systems in anti-tumour immunity has become apparent. The key effector subsets driving immunotherapy response may differ depending on cancer type, as evidenced by Zeverijn et al., who found innate immunity to be more important in microsatellite instable (MSI) tumours compared to melanoma[108]. Due to the direct link between these innate(-like) immune subsets and pathogen defence, it is likely that changes in the microbiome will have an impact on the functionality of these cells, which may in turn affect anti-tumour immunity. Finally, other cells within the TME, including cancer-associated fibroblasts and other stromal cells, may also be influenced by microbiome-metabolites, a possibility that remains largely underexplored. Therefore, human datasets and model systems are crucial to uncover how diverse cell types contribute to microbiome-driven immune modulation.

In summary, several important questions remain: (1) which metabolites are most essential for anti-tumour immunity; (2) are we identifying all relevant metabolites; (3) are these effects context dependent; (4) how can we best utilise these microbial metabolites for therapy; and (5) what are optimal combination strategies. Furthermore, despite the techniques currently available, microbiome research remains challenging. Advancements in technologies, as well as building large multi-omics clinical datasets, should be a priority for the future to drive the field forward. Ultimately, these approaches will help the development of microbiota-targeting therapies that enhance anti-tumour immunity, thereby improving the quality of life and survival of patients.

References

1. Gaynor, N., Crown, J. & Collins, D. M. Immune checkpoint inhibitors: key trials and an emerging role in breast cancer. *Semin. Cancer Biol.* **79**, 44–57 (2022).
2. Das, S. & Johnson, D. B. Immune-related adverse events and anti-tumor efficacy of immune checkpoint inhibitors. *J. Immunother. Cancer* **7**, 306 (2019).
3. Hanahan, D. Hallmarks of cancer: new dimensions. *Cancer Discov.* **12**, 31–46 (2022).
4. Wilson, B. E., Routy, B., Nagrial, A. & Chin, V. T. The effect of antibiotics on clinical outcomes in immune-checkpoint blockade: a systematic review and meta-analysis of observational studies. *Cancer Immunol. Immunother.* **69**, 343–354 (2020).
5. Elkrief, A. et al. Antibiotics are associated with worse outcomes in lung cancer patients treated with chemotherapy and immunotherapy. *NPJ Precis Oncol.* **8**, 143 (2024).
6. Routy, B. et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science* **359**, 91–97 (2018).
7. Sivan, A. et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **350**, 1084–1089 (2015).
8. Gopalakrishnan, V. et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **359**, 97–103 (2018).
9. Lee, K. A. et al. Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma. *Nat. Med.* **28**, 535–544 (2022).
10. Davar, D. et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* **371**, 595–602 (2021).
11. Baruch, E. N. et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* **371**, 602–609 (2021).
12. Routy, B. et al. Fecal microbiota transplantation plus anti-PD-1 immunotherapy in advanced melanoma: a phase I trial. *Nat. Med.* **29**, 2121–2132 (2023).
13. Tomita, Y. et al. Association of probiotic clostridium butyricum therapy with survival and response to immune checkpoint blockade in patients with lung cancer. *Cancer Immunol. Res.* **8**, 1236–1242 (2020).

14. Takada, K. et al. Clinical impact of probiotics on the efficacy of anti-PD-1 monotherapy in patients with nonsmall cell lung cancer: a multicenter retrospective survival analysis study with inverse probability of treatment weighting. *Int. J. Cancer* **149**, 473–482 (2021).
15. Dizman, N. et al. Nivolumab plus ipilimumab with or without live bacterial supplementation in metastatic renal cell carcinoma: a randomized phase 1 trial. *Nat. Med.* **28**, 704–712 (2022).
16. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
17. Gilbert, J. A. et al. Current understanding of the human microbiome. *Nat. Med.* **24**, 392–400 (2018).
18. Li, C., Liang, Y. & Qiao, Y. Messengers from the gut: gut microbiota-derived metabolites on host regulation. *Front. Microbiol* **13**, 863407 (2022).
19. Krautkramer, K. A., Fan, J. & Bäckhed, F. Gut microbial metabolites as multi-kingdom intermediates. *Nat. Rev. Microbiol.* **19**, 77–94 (2021).
20. Pascal Andreu, V. et al. gutSMASH predicts specialized primary metabolic pathways from the human gut microbiota. *Nat. Biotechnol.* **41**, 1416–1423 (2023).
21. Hezaveh, K. et al. Tryptophan-derived microbial metabolites activate the aryl hydrocarbon receptor in tumor-associated macrophages to suppress anti-tumor immunity. *Immunity* **55**, 324–340 e328 (2022).
22. Mirji, G. et al. The microbiome-derived metabolite TMAO drives immune activation and boosts responses to immune checkpoint blockade in pancreatic cancer. *Sci. Immunol.* **7**, eabn0704 (2022).
23. Nastasi, C. et al. The effect of short-chain fatty acids on human monocyte-derived dendritic cells. *Sci. Rep.* **5**, 16148 (2015).
24. Coutzac, C. et al. Systemic short chain fatty acids limit antitumor effect of CTLA-4 blockade in hosts with cancer. *Nat. Commun.* **11**, 2168 (2020).
25. Perez, M., Buey, B., Corral, P., Giraldos, D. & Latorre, E. Microbiota-derived short-chain fatty acids boost antitumoral natural killer cell activity. *J. Clin. Med.* **13**, 3885 (2024).
26. Liu, N. et al. *Eubacterium rectale* improves the efficacy of anti-PD1 immunotherapy in melanoma via L-serine-mediated NK cell activation. *Research* **6**, 0127 (2023).
27. Maddocks, O. D. K. et al. Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells. *Nature* **493**, 542–546 (2013).
28. Maddocks, O. D. K. et al. Modulating the therapeutic response of tumours to dietary serine and glycine starvation. *Nature* **544**, 372–376 (2017).
29. Gungabeesoon, J. et al. A neutrophil response linked to tumor control in immunotherapy. *Cell* **186**, 1448–1464.e1420 (2023).
30. Wu, Y. et al. Neutrophil profiling illuminates anti-tumor antigen-presenting potency. *Cell* **187**, 1422–1439.e1424 (2024).
31. Blomberg, O. S. et al. IL-5-producing CD4+ T cells and eosinophils cooperate to enhance response to immune checkpoint blockade in breast cancer. *Cancer Cell* **41**, 106–123.e110 (2023).
32. Ribot, J. C., Lopes, N. & Silva-Santos, B. gammadelta T cells in tissue physiology and surveillance. *Nat. Rev. Immunol.* **21**, 221–232 (2021).
33. Jabeen, M. F. & Hinks, T. S. C. MAIT cells and the microbiome. *Front. Immunol.* **14**, 1127588 (2023).
34. Vogt, S. & Mattner, J. NKT cells contribute to the control of microbial infections. *Front. Cell Infect. Microbiol* **11**, 718350 (2021).
35. Ruf, B., Greten, T. F. & Korangy, F. Innate lymphoid cells and innate-like T cells in cancer—at the crossroads of innate and adaptive immunity. *Nat. Rev. Cancer* **23**, 351–371 (2023).
36. Hintz, M. et al. Identification of (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate as a major activator for human gammadelta T cells in *Escherichia coli*. *FEBS Lett.* **509**, 317–322 (2001).
37. Holmen Olofsson, G. et al. Vgamma9Vdelta2 T cells concurrently kill cancer cells and cross-present tumor antigens. *Front. Immunol.* **12**, 645131 (2021).
38. Mimpen, I. L. et al. Microbial metabolic pathways guide response to immune checkpoint blockade therapy. *Cancer Discov.* **16**, 95–113 (2025).
39. Kjer-Nielsen, L. et al. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* **491**, 717–723 (2012).
40. Eckle, S. B. et al. Recognition of vitamin B precursors and byproducts by mucosal associated invariant T Cells. *J. Biol. Chem.* **290**, 30204–30211 (2015).
41. Li, S. et al. Human Tumor-infiltrating MAIT cells display hallmarks of bacterial antigen recognition in colorectal cancer. *Cell Rep. Med.* **1**, 100039 (2020).
42. Ma, C. et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* **360**, eaan5931 (2018).
43. Adams, E. J., Gu, S. & Luoma, A. M. Human gamma delta T cells: evolution and ligand recognition. *Cell Immunol.* **296**, 31–40 (2015).
44. Mestas, J. & Hughes, C. C. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* **172**, 2731–2738 (2004).
45. Salou, M., Legoux, F. & Lantz, O. MAIT cell development in mice and humans. *Mol. Immunol.* **130**, 31–36 (2021).
46. Phelps, C. M. et al. Exercise-induced microbiota metabolite enhances CD8 T cell antitumor immunity promoting immunotherapy efficacy. *Cell* **188**, 5680–5700 (2025).
47. Bender, M. J. et al. Dietary tryptophan metabolite released by intratumoral *Lactobacillus reuteri* facilitates immune checkpoint inhibitor treatment. *Cell* **186**, 1846–1862 e1826 (2023).
48. Marcos-Kovandzic, L. et al. Gut microbiota modulation through akkermansia spp. supplementation increases CAR T-cell potency. *Cancer Discov.* **15**, 1905–1926 (2025).
49. Jin, W. B. et al. Microbiota-derived bile acids antagonize the host androgen receptor and drive anti-tumor immunity. *Cell* **188**, 2336–2353 e2338 (2025).
50. Varanasi, S. K. et al. Bile acid synthesis impedes tumor-specific T cell responses during liver cancer. *Science* **387**, 192–201 (2025).
51. Luu, M. et al. Microbial short-chain fatty acids modulate CD8(+) T cell responses and improve adoptive immunotherapy for cancer. *Nat. Commun.* **12**, 4077 (2021).
52. Wang, X. et al. Fusobacterium nucleatum facilitates anti-PD-1 therapy in microsatellite stable colorectal cancer. *Cancer Cell* **42**, 1729–1746 e1728 (2024).
53. Zhu, X. et al. Microbial metabolite butyrate promotes anti-PD-1 antitumor efficacy by modulating T cell receptor signaling of cytotoxic CD8 T cell. *Gut Microbes* **15**, 2249143 (2023).
54. Jia, D. et al. Microbial metabolite enhances immunotherapy efficacy by modulating T cell stemness in pan-cancer. *Cell* **187**, 1651–1665 e1621 (2024).
55. Mager, L. F. et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* **369**, 1481–1489 (2020).
56. Kang, X. et al. Roseburia intestinalis generated butyrate boosts anti-PD-1 efficacy in colorectal cancer by activating cytotoxic CD8(+) T cells. *Gut* **72**, 2112–2122 (2023).
57. Zhu, X. et al. Interplay between gut microbial communities and metabolites modulates pan-cancer immunotherapy responses. *Cell Metab.* **37**, 806–823 e806 (2025).
58. Hazekawa, M., Ono, K., Nishinakagawa, T., Kawakubo-Yasukochi, T. & Nakashima, M. In vitro anti-inflammatory effects of the

phenylbutyric acid metabolite phenylacetyl glutamine. *Biol. Pharm. Bull.* **41**, 961–966 (2018).
59. Denk, D. et al. Expansion of T memory stem cells with superior anti-tumor immunity by Urolithin A-induced mitophagy. *Immunity* **55**, 2059–2073 e2058 (2022).
60. St Paul, M. et al. Coenzyme A fuels T cell anti-tumor immunity. *Cell Metab.* **33**, 2415–2427 e2416 (2021).
61. Uribe-Herranz, M. et al. Microbiota shape metabolic and immune determinants of CAR-T therapy and correlate with outcomes in myeloma. *Blood Cancer Discov.* **6**, 484–504 (2025).
62. Shen, Y. et al. Ursodeoxycholic acid reduces antitumor immunosuppression by inducing CHIP-mediated TGF-beta degradation. *Nat. Commun.* **13**, 3419 (2022).
63. Zhang, L. et al. Inhibition of UBA6 by inosine augments tumour immunogenicity and responses. *Nat. Commun.* **13**, 5413 (2022).
64. Ferrari, V. et al. Sensitizing cancer cells to immune checkpoint inhibitors by microbiota-mediated upregulation of HLA class I. *Cancer Cell* **41**, 1717–1730 e1714 (2023).
65. Zhang, J. et al. Targeting gut microbiota and arginase boosts MEK inhibitors' enhancement of antitumour immunity via MHC-I upregulation in colorectal cancer. *Br. J. Cancer* **133**, 809–822 (2025).
66. Teng, Y. et al. Plant-nanoparticles enhance anti-PD-L1 efficacy by shaping human commensal microbiota metabolites. *Nat. Commun.* **16**, 1295 (2025).
67. Wang, B. et al. *Blautia coccoides* and its metabolic products enhance the efficacy of bladder cancer immunotherapy by promoting CD8(+) T cell infiltration. *J. Transl. Med.* **22**, 964 (2024).
68. Zhang, M. et al. Microbiota-derived urocanic acid triggered by tyrosine kinase inhibitors potentiates cancer immunotherapy efficacy. *Cell Host Microbe* **33**, 915–931 e919 (2025).
69. Jiang, S. S. et al. Fusobacterium nucleatum-derived succinic acid induces tumor resistance to immunotherapy in colorectal cancer. *Cell Host Microbe* **31**, 781–797 e789 (2023).
70. Wang, H. et al. The microbial metabolite trimethylamine N-oxide promotes antitumor immunity in triple-negative breast cancer. *Cell Metab.* **34**, 581–594 e588 (2022).
71. Yu, P. et al. Pyroptosis: mechanisms and diseases. *Signal Transduct. Target Ther.* **6**, 128 (2021).
72. Sardar, P., Almeida, A. & Pedicord, V. A. Integrating functional metagenomics to decipher microbiome-immune interactions. *Immunol. Cell Biol.* **102**, 680–691 (2024).
73. Bauermeister, A., Mannochio-Russo, H., Costa-Lotufo, L. V., Jarmusch, A. K. & Dorrestein, P. C. Mass spectrometry-based metabolomics in microbiome investigations. *Nat. Rev. Microbiol.* **20**, 143–160 (2022).
74. Smirnov, K. S. et al. Challenges of metabolomics in human gut microbiota research. *Int. J. Med Microbiol.* **306**, 266–279 (2016).
75. Sun, Y. et al. Integrative plasma and fecal metabolomics identify functional metabolites in adenoma-colorectal cancer progression and as early diagnostic biomarkers. *Cancer Cell* **42**, 1386–1400.e1388 (2024).
76. Mirzayi, C. et al. Reporting guidelines for human microbiome research: the STORMS checklist. *Nat. Med.* **27**, 1885–1892 (2021).
77. Action, H. M. <https://humanmicrobiomeaction.eu> (
78. Ferri-Borgogno, S. et al. Molecular, metabolic, and subcellular mapping of the tumor immune microenvironment via 3D targeted and non-targeted multiplex multi-omics analyses. *Cancers* **16**, 846 (2024).
79. Alexandrov, T. Spatial metabolomics: from a niche field towards a driver of innovation. *Nat. Metab.* **5**, 1443–1445 (2023).
80. Allen, T. M. et al. Humanized immune system mouse models: progress, challenges and opportunities. *Nat. Immunol.* **20**, 770–774 (2019).
81. Hugenholtz, F. & de Vos, W. M. Mouse models for human intestinal microbiota research: a critical evaluation. *Cell Mol. Life Sci.* **75**, 149–160 (2018).
82. Day, C. P., Merlino, G. & Van Dyke, T. Preclinical mouse cancer models: a maze of opportunities and challenges. *Cell* **163**, 39–53 (2015).
83. Park, J. C. & Im, S.-H. Of men in mice: the development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. *Exp. Mol. Med.* **52**, 1383–1396 (2020).
84. Shelkey, E. et al. Immuno-reactive cancer organoid model to assess effects of the microbiome on cancer immunotherapy. *Sci. Rep.* **12**, 9983 (2022).
85. Puschhof, J. et al. Intestinal organoid cocultures with microbes. *Nat. Protoc.* **16**, 4633–4649 (2021).
86. Ballerini, M. et al. A gut-on-a-chip incorporating human faecal samples and peristalsis predicts responses to immune checkpoint inhibitors for melanoma. *Nat. Biomed. Eng.* **9**, 967–984 (2025).
87. Shin, Y. C., Than, N., Min, S., Shin, W. & Kim, H. J. Modelling host–microbiome interactions in organ-on-a-chip platforms. *Nat. Rev. Bioeng.* **2**, 175–191 (2024).
88. Voabil, P. et al. An ex vivo tumor fragment platform to dissect response to PD-1 blockade in cancer. *Nat. Med* **27**, 1250–1261 (2021).
89. Spencer, C. N. et al. Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science* **374**, 1632–1640 (2021).
90. Simpson, R. C. et al. Diet-driven microbial ecology underpins associations between cancer immunotherapy outcomes and the gut microbiome. *Nat. Med.* **28**, 2344–2352 (2022).
91. Farias, R. M. et al. Diet and immune effects trial (DIET)—a randomized, double-blinded dietary intervention study in patients with melanoma receiving immunotherapy. *BMC Cancer* **24**, 1493 (2024).
92. Messaoudene, M. et al. A natural polyphenol exerts antitumor activity and circumvents anti-PD-1 resistance through effects on the gut microbiota. *Cancer Discov.* **12**, 1070–1087 (2022).
93. Han, K. et al. Generation of systemic antitumour immunity via the in situ modulation of the gut microbiome by an orally administered inulin gel. *Nat. Biomed. Eng.* **5**, 1377–1388 (2021).
94. Zhang, C. et al. Mannose enhances immunotherapy efficacy in ovarian cancer by modulating gut microbial metabolites. *Cancer Res.* **85**, 2468–2484 (2025).
95. da Silva, I. P. et al. Retreatment, rechallenge, and escalation with subsequent immune checkpoint inhibitor therapies across cancers after initial failure. *ESMO Open* **10**, 105833 (2025).
96. Canale, F. P. et al. Metabolic modulation of tumours with engineered bacteria for immunotherapy. *Nature* **598**, 662–666 (2021).
97. Vaaben, T. H. et al. Modulating tumor immunity using advanced microbiome therapeutics producing an indole metabolite. *EMBO Rep.* **26**, 1688–1708 (2025).
98. Bignaud, A. et al. Phages with a broad host range are common across ecosystems. *Nat. Microbiol.* **10**, 2537–2549 (2025).
99. Wirbel, J. et al. Long-read metagenomics reveals phage dynamics in the human gut microbiome. *Nature* **649**, 982–990 (2025).
100. Jault, P. et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas* aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect. Dis.* **19**, 35–45 (2019).
101. Petrovic Fabijan, A. et al. Safety of bacteriophage therapy in severe *Staphylococcus aureus* infection. *Nat. Microbiol.* **5**, 465–472 (2020).
102. Ding, X. et al. Bacteroides fragilis promotes chemoresistance in colorectal cancer, and its elimination by phage VA7 restores chemosensitivity. *Cell Host Microbe* **33**, 941–956 e910 (2025).

103. Zheng, D. W. et al. Phage-guided modulation of the gut microbiota of mouse models of colorectal cancer augments their responses to chemotherapy. *Nat. Biomed. Eng.* **3**, 717–728 (2019).
104. Cong, J. et al. Bile acids modified by the intestinal microbiota promote colorectal cancer growth by suppressing CD8(+) T cell effector functions. *Immunity* **57**, 876–889 e811 (2024).
105. Hsu, B. B. et al. Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. *Cell Host Microbe* **25**, 803–814 e805 (2019).
106. Shen, J. et al. Large-scale phage cultivation for commensal human gut bacteria. *Cell Host Microbe* **31**, 665–677 e667 (2023).
107. Seifert, M. et al. Impact of the selective A2(A)R and A2(B)R dual antagonist AB928/etrumadenant on CAR T cell function. *Br. J. Cancer* **127**, 2175–2185 (2022).
108. Zeverijn, L. J. et al. The innate immune landscape of dMMR/MSI cancers predicts the outcome of nivolumab treatment: results from the drug rediscovery protocol. *Clin. Cancer Res.* **30**, 4339–4351 (2024).
109. Koh, A., De Vadder, F., Kovatcheva-Datchary, P. & Backhed, F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* **165**, 1332–1345 (2016).
110. Garcia-Villalba, R. et al. Urolithins: a comprehensive update on their metabolism, bioactivity, and associated gut microbiota. *Mol. Nutr. Food Res.* **66**, e2101019 (2022).
111. Tennoune, N., Andriamihaja, M. & Blachier, F. Production of indole and indole-related compounds by the intestinal microbiota and consequences for the host: the good, the bad, and the ugly. *Microorganisms* **10**, 930 (2022).
112. Fleishman, J. S. & Kumar, S. Bile acid metabolism and signaling in health and disease: molecular mechanisms and therapeutic targets. *Signal Transduct. Target Ther.* **9**, 97 (2024).
113. Martinez-del Campo, A. et al. Characterization and detection of a widely distributed gene cluster that predicts anaerobic choline utilization by human gut bacteria. *mBio* **6**, e00042-15 (2015).
114. Wu, W. K. et al. Gut microbes with the gbu genes determine TMAO production from L-carnitine intake and serve as a biomarker for precision nutrition. *Gut Microbes* **17**, 2446374 (2025).
115. Nemet, I. et al. A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors. *Cell* **180**, 862–877 e822 (2020).
116. Bottcher, J., Sibon, O. C. M. & El Aidy, S. Coenzyme A metabolism: a key driver of gut microbiota dynamics and metabolic profiles. *FEMS Microbiol. Rev.* **49**, fuaf051 (2025).
117. Javdan, B. et al. Personalized mapping of drug metabolism by the human gut microbiome. *Cell* **181**, 1661–1679 e1622 (2020).
118. Fidelle, M. et al. A microbiota-modulated checkpoint directs immunosuppressive intestinal T cells into cancers. *Science* **380**, eabo2296 (2023).

Acknowledgements

We sincerely acknowledge Mrs Anneke Hoogendijk and the Foundation Weteringschans for their generous support, which has enabled our investigation of the role of the microbiome in cancer immunotherapy responses.

Author contributions

C. Toner-Bartelds and I. L. Mimpen contributed equally to the preparation of this manuscript. C. Toner-Bartelds: conceptualisation, investigation, writing—original draft preparation, review, and editing. I. L. Mimpen: conceptualisation, investigation, writing—original draft preparation, review, and editing. M. Parra-Martinez: investigation, writing—original draft preparation, review, and editing. B. M. T. Burgering: assistance with revisions and writing—review. E. Voest: conceptualisation, supervision, funding acquisition and writing—review and editing.

Competing interests

E. Voest is the founder and current member of the supervisory board of the Hartwig Medical Foundation, an independent non-executive director and shareholder of Sanofi, a co-founder and shareholder of Mosaic Therapeutics, and a board member and founder of the Centre for Personalised Cancer Treatment. He has received clinical study grants from Amgen, AstraZeneca, Bayer, BMS, Boehringer Ingelheim, Clovis, Eisai, Eli Lilly, GSK, Ipsen, Johnson & Johnson, MSD, Novartis, Pfizer, Roche, Servier, Incyte, MRM Health and Sanofi, all paid to the Netherlands Cancer Institute. C. Toner-Bartelds, I. Mimpen, M. Parra-Martinez and B. Burgering declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Emile E. Voest.

Peer review information *Nature Communications* thanks the anonymous reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2026