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The gut microbiome and cancer: from tumorigenesis to therapy

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The gut microbiome has a crucial role in cancer development and therapy through its interactions with the immune system and tumour microenvironment. Although evidence links gut microbiota composition to cancer progression, its precise role in modulating treatment responses remains unclear. In this Review, we summarize current knowledge on the gut microbiome's involvement in cancer, covering its role in tumour initiation and progression, interactions with chemotherapy, radiotherapy and targeted therapies, and its influence on cancer immunotherapy. We discuss the impact of microbial metabolites on immune responses, the relationship between specific bacterial species and treatment outcomes, and potential microbiota-based therapeutic strategies, including dietary interventions, probiotics and faecal microbiota transplantation. Understanding these complex microbiota–immune interactions is critical for optimizing cancer therapies. Future research should focus on defining microbial signatures associated with treatment success and developing targeted microbiome modulation strategies to enhance patient outcomes.

The human microbiome is defined as the collection of microorganisms, encompassing their genetic material and metabolic by-products, that inhabit the human body from birth. Although microbial communities colonize all surfaces exposed to the external environment, the gastrointestinal tract harbours the highest microbial density and has been the subject of extensive research. Evidence shows that the gut microbiome vastly surpasses the human genome with the ratio of microbial to human cells estimated to be approximately 100:1 (ref. 1). This considerable difference underscores the profound impact that gut microorganisms potentially exert on their host.

Over the past two decades, numerous discoveries have elucidated how the gut microbiota influence human health[2,3]. These microbial communities actively interact with their hosts through various mechanisms, which include the metabolization and fermentation of diverse compounds resulting in the production of bioactive metabolites, regulation of gut barrier integrity and modulation of the immune system[4,5]. The gut microbiota composition and functionality exhibit considerable interindividual variability, influenced by factors such as diet, genetics, age, lifestyle, environment and medication[3,6]. In addition, technical aspects should also be considered (Box 1). These diverse influences contribute to the complexity of defining a 'normal' or healthy gut microbiota profile[7]. Nevertheless, disturbances or imbalances in the gut microbiota, often characterized by reduced microbial diversity, the loss of commensal bacteria and an overgrowth of pathogenic species (often inaccurately referred to as 'dysbiosis') have been implicated in the progression of numerous immune-mediated

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BOX 1

Factors influencing reproducibility of microbiota–cancer results

Although variability between individuals is a well-recognized challenge, variability between studies often introduces important barriers to reproducibility, as highlighted in recent studies[20,292]. Studies should further emphasize the importance of methodological standardization and comprehensive experimental design. Technical variability, for example, can arise from differences in methodologies and platforms, including sequencing depth, sample preservation methods and DNA extraction protocols[293,294]. In some cases, technical variability has been shown to exceed biological variability, which underscores its impact on the reliability of microbiome research. Microbiome compositions within the same individual can fluctuate over time owing to changes in diet, medication or health status. Moreover, species-level and strain-level differences represent an often underappreciated yet substantial source of variability. Microbiome compositions can differ not only across species but also at the strain and substrain levels, with functional implications that are increasingly recognized as important for understanding host–microbiome interactions. With advancements in microbiome analysis tools, such as strain-level resolution in metagenomics[295], these differences are becoming more discernible and should be accounted for in study designs and interpretations.

Although the primary focus of the Review may not be on technological considerations, it is valuable to mention the advancements and limitations of microbiome research tools. For example, 16S rRNA gene sequencing is a widely used and cost-effective method for profiling microbial communities in stool and tissue samples. However, its limited taxonomic resolution and inability to provide functional insights are notable drawbacks. Metagenomics offers species-level resolution and functional potential by sequencing entire microbial genomes but requires high-quality DNA and important computational resources. Similarly, metatranscriptomics, which captures actively transcribed genes, offers insights into microbial function but demands stringent protocols to avoid RNA degradation. Emerging spatial omics technologies, such as spatial transcriptomics, are particularly promising for integrating spatial resolution with functional profiling, enabling unprecedented insights into microbe–host interactions at the tissue level. However, these methods remain in their infancy and face challenges related to accessibility and scalability.

conditions throughout the body, such as inflammatory bowel disease, asthma, allergies and diabetes.

Beyond its role in metabolic and immune homeostasis, the gut microbiota has emerged as a key player in cancer development and progression[8–10] (Fig. 1). Microorganisms regulate immune responses at both local and systemic levels, influencing inflammation, immune surveillance and tolerance. A well-balanced microbiota supports effective immune function, but disruptions—driven by diet, environmental factors, lifestyle, infections or medications—can promote chronic inflammation and impair antitumour immunity. Increasing evidence suggests that these disturbances contribute not only to tumour initiation and progression but also to resistance to therapy. As a result, the gut microbiota is now recognized as an important factor shaping cancer risk and treatment outcomes.

The mechanisms driving these interactions represent an exciting and rapidly evolving area of research in cancer biology and therapeutic development. This Review explains the latest discoveries on the mechanisms through which distinct bacterial species or their metabolites influence cancer development. The recent findings regarding the fundamentals of cancer immunotherapy and how microbiota–immune interactions have a role in cancer are covered in this Review. Recent advancements on connections and mechanisms potentially leading the complex relationships of cancers and gut microorganisms are discussed. Finally, potential manipulation strategies aimed at enhancing therapeutic outcomes for individuals with cancer are presented.

Microbiome, cancer formation and progression

Cancer is a genetic disease arising from the stepwise accumulation of (epi)genomic alterations impacting the normal behaviour of tumour-suppressor genes and oncogenes. It is the second leading cause of death worldwide, diagnosed in approximately one in five humans in their lifetime[11,12]. Most cases arise from somatically acquired genomic alterations, rather than from hereditary factors. Among the carcinogens, chemical, physical, biological and lifestyle factors collectively are the most studied, as reviewed in the World Health Organization (WHO) International Agency for Research on Cancer monographs (http://monographs.iarc.fr/). The incidence of early-onset cancers has been increasing across multiple countries in recent decades[13]. Evidence points to risk factor exposures during early life and young adulthood as key contributors to this trend. In addition to changes in diet, lifestyle, obesity and environmental factors, the role of the gut microbiome has gained extensive attention. Notably, many of the early-onset cancer types with rising incidence are linked to the digestive system[13].

Increasing evidence furthermore suggests the association between deviations of the gut microbiota and the development and progression of cancer at multiple anatomical sites, both adjacent and distal to the microbial localization[14]. The causality of specific microorganisms is now demonstrated for several cancer types, with metabolic by-products seeming to have key roles, mainly impacting gatekeeper cellular components and antitumour immunity[15,16]. In the next section, we will specifically describe cancer types for which the occurrence and progression have been related to gut microbiota deviation.

Changes in microbiota composition

Changes in gut microbiota composition in gastrointestinal cancers. *Colorectal cancer*. The development of colorectal cancer (CRC) is closely linked to interactions between various microorganisms, their metabolites and the colonic epithelium (see recent systematic reviews[17,18]). Studies in animal models and cell cultures have shown that specific microorganisms, such as enterotoxigenic *Bacteroides fragilis* (ETBF), *Fusobacterium nucleatum* and *Escherichia coli* carrying the polyketide synthase island (*pks*$^+$ *E. coli*) contribute to the tumorigenesis. Experiments involving faecal microbiota transplantation (FMT) from individuals with CRC into germ-free mice have demonstrated significant effects, including increased cell proliferation, a higher number of polyps, greater dysplasia and elevated inflammatory markers compared to transplants from healthy donors[19]. These findings strongly suggest that gut microbiota imbalance may be a causative factor in CRC development. Establishing causation in humans, however, remains difficult owing to the multifactorial nature of CRC, which involves genetic, epigenetic, environmental and microbial factors. Additional challenges include the lack of large cohort studies with long-term follow-up and inconsistencies in sampling and analysis methods[20]. Nonetheless, elevated levels of some specific bacteria are consistently found in CRC tissues compared to normal or benign lesions[21,22], and some studies provide strong in vitro or in vivo mechanistic evidence of causality between gut bacteria and occurrence of oncogenic somatic mutations.

ETBF is recognized as an important 'alpha-bug' due to its strong pro-tumorigenic properties. An alpha-bug is a microorganism that

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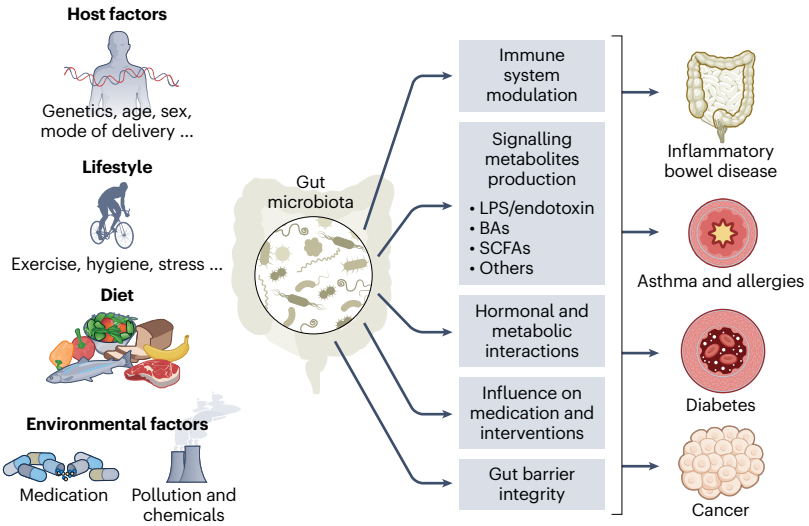

Fig. 1 | Gut microbiota in general, main metabolites produced and their targets. This figure illustrates the impact of various environmental factors (host factors, lifestyle, diet, medication and pollution) on gut microbiota. The gut microbiota modulates immune function, produces signalling metabolites (for example, LPS/endotoxin, BAs and SCFAs), influences hormonal and metabolic interactions, affects medication responses and maintains gut barrier integrity. These interactions are linked to health outcomes, such as inflammatory bowel disease, asthma and allergies, diabetes, and cancer.

exerts a disproportionate influence on its environment, including the host's immune system, local microbiota and the progression of disease. It is not necessarily the most abundant organism but has a substantial functional impact.

This aligns with the 'driver–passenger' model, which suggests that alpha-bugs such as ETBF act as 'drivers' by initiating tumorigenic changes in the microenvironment through chronic inflammation, epithelial barrier disruption and host signalling modulation[23]. These alterations create a permissive environment that allows commensal 'passenger' bacteria, such as *F. nucleatum*, to colonize and exacerbate tumour progression[24,25]. *F. nucleatum* expresses the virulence factor fusobacterial adhesin A on its cell surface, which directly interacts with epithelial E-cadherin to activate β-catenin signalling. This signalling cascade, through Toll-like receptor (TLR) 4 activation, enhances tumour cell proliferation and survival, promoting cancer progression[26]. Additionally, *F. nucleatum* has been implicated in chemotherapy resistance through stimulation of autophagy-related proteins in tumour cells, which can for example counteract the cytotoxic effects of chemotherapeutic agents[25]. The clinical importance of *F. nucleatum* is further underscored by its association with patient outcomes[27]. Higher levels of *F. nucleatum* DNA detected in CRC tumour tissues correlate with increased CRC-specific mortality, suggesting its potential utility as a prognostic biomarker. Its persistence in the tumour microenvironment, even after interventions such as chemotherapy, has also been linked to increased rates of relapse, reinforcing its critical role in CRC pathogenesis and progression[28].

The genotoxic capabilities of *E. coli* strains carrying the *pks*$^+$ pathogenicity island have been highlighted in CRC research[29]. The *pks*$^+$ island encodes a series of enzymes responsible for synthesizing colibactin, a potent genotoxin known to cause direct DNA damage in epithelial cells. This damage is primarily characterized by the induction of DNA crosslinks and double-strand breaks, leading to genomic instability, which is a hallmark of cancer progression. Colibactin-associated mutations have been identified in more than 12% of CRC cases[30,31], underscoring its substantial contribution to the disease burden. Co-culture of human intestinal organoids and *pks*$^+$ *E. coli* strains allowed the definition of mutagenic events induced by these bacteria. *pks*$^+$-specific mutational signatures could be derived, and the authors showed their appearance was causally related to colibactin exposure[32]. Further studies enhanced our understanding of the mutagenic activity of *pks*$^+$ *E. coli* strains, demonstrating colibactin induces specific mutations enriched in CRC oncogenesis, including mutations in *APC*, which is a pivotal tumour-suppressor gene in this disease[30]. Intriguingly, these colibactin-induced mutational events have also been associated with earlier-onset CRC cases[30].

Furthermore, other bacterial species such as *Peptostreptococcus anaerobius* and *Peptostreptococcus stomatis* have emerged as notable contributors to CRC. *P. anaerobius* has a role in creating an immunosuppressive tumour microenvironment by inducing the recruitment and activation of intratumoural myeloid-derived suppressor cells (MDSCs). This process is mediated through the chemokine CXCL1, which is produced by tumour cells in response to bacterial colonization. MDSCs suppress T cell activity, thereby dampening antitumour immune responses and significantly reducing the efficacy of anti-programmed cell death 1 (PD-1) immune checkpoint blockade therapies in CRC models[33]. This highlights the potential of *P. anaerobius* as a therapeutic target to enhance the efficacy of immunotherapies. Recently, a metabolite produced by *P. anaerobius* has been shown to promote CRC by inhibition of ferroptosis[34]. Supplementation with this metabolite or *P. anaerobius* accelerated CRC progression in mouse models[34]. Similarly, *P. stomatis* has demonstrated the capacity to exacerbate tumorigenesis in carcinogen-induced mouse models of CRC. Its presence has been linked to an increased tumour burden, higher incidences of high-grade dysplasia and adenocarcinoma formation. Mechanistically, *P. stomatis* undermines gut barrier integrity, promotes a pro-inflammatory state and enhances cell proliferation. These effects are driven by its activation of the oncogenic mitogen-activated protein kinase (MAPK) signalling pathway through the ERBB2 receptor, which is a regulator of cell growth and differentiation[35].

By contrast, bacteria generally regarded as 'beneficial,' including *Bifidobacterium*, *Clostridium butyricum*, *Faecalibacterium prausnitzii* and *Roseburia intestinalis*, are consistently found in reduced abundance in stools of individuals with CRC[36]. These microorganisms contribute to intestinal health through the production of metabolites, such as short-chain fatty acids (SCFAs), which have a protective role against CRC. They have been shown to dampen cell proliferation, induce apoptosis in cancer cells and modulate immune responses, thereby maintaining epithelial integrity and mitigating tumour progression. More specifically, *C. butyricum* seems able to inhibit the development of colon cancer, through suppression of the Wnt pathway

in colon cancer cells and secondary modulation of the gut microbiota composition, inducing higher rates of SCFA-producing bacteria[37]. *C. butyricum* putatively exerts protective effects against colitis and prevents secondary colon cancer through the same mechanisms[38]. The role of these microbial metabolites, along with their mechanisms of action in cancer prevention and progression, will be explored in greater detail later in this Review.

More studies have connected imbalances in gut microbiota with the promotion of metastatic CRC by shaping a metastatic niche and inducing the epithelial–mesenchymal transition (for recent reviews, see refs. 39,40). For example, in antibiotic-treated mice with administration of *E. coli* to mimic gut microbiota imbalance, elevated levels of lipopolysaccharide (LPS) and cathepsin K, a metastasis-related secretory protein, overexpression promote the aggressiveness of CRC. Cathepsin K interacts with the TLR4 receptor to activate the mTOR pathway, in turn stimulating tumour-associated macrophages to promote CRC invasion. Cathepsin K could be a biomarker as it correlated in human CRC tissue with CRC metastasis and poor prognosis[41]. Studies comparing gut microbiota with the microbiota in metastatic sites have identified shared species between primary colorectal tumours and distant metastases, such as those in the liver. Notably, *F. nucleatum* was consistently detected in both primary and metastatic lesions, indicating that the microbiota may maintain its composition during the metastatic process[27]. This finding suggests a potential role for *F. nucleatum* in facilitating the dissemination of CRC cells, possibly through mechanisms involving immune modulation or direct microbial support of metastatic niches.

Advances in microbiome profiling technologies, such as metagenomics and transcriptomics, along with functional studies in human organoid models and humanized mice, could pave the way for a deeper understanding of the mechanistic roles played by bacteria in CRC. For instance, a comprehensive pangenome analysis of *F. nucleatum* has provided insights into strain-to-strain variations in its genotypic and phenotypic characteristics[21]. This study identified a subset of bacterial strains with enhanced carcinogenic potential, suggesting that only certain strains possess the genetic repertoire necessary to establish themselves in the CRC microenvironment. Strains enriched in the tumour were found to exhibit increased metabolic potential, including enhanced nutrient scavenging capabilities and elevated oxidative stress levels, both of which could facilitate their survival and colonization within the tumour microenvironment[21]. These findings underline the importance of focusing on the functional diversity of bacterial strains rather than treating microbial species as uniform entities. Such insights could inform targeted therapies and diagnostic approaches, emphasizing the interplay between microbial adaptation and tumour progression.

Gastric cancer. Analysis of gastric mucosal samples has shown differences in the microbiota composition across the stages of gastric disease, from superficial gastritis to atrophic gastritis, gastric metaplasia and ultimately gastric cancer (GC)[42,43] compared to healthy individuals[44]. Enrichment of specific lactic acid-producing bacteria such as *Lactococcus* and *Lactobacillus* and enrichment of SCFA production pathways have been highlighted in gastric biopsy samples from individuals with GC[44]. Besides these specific bacterial signatures, *Helicobacter pylori* infection is recognized as a key factor for the development of GC and associated mortality[45,46]. *H. pylori* infection tends to dominate the gastric niche, harbouring a significantly lower diversity of bacteria in the stomach[47]. This bacterium, considered as a class I carcinogen, uses virulence factors such as cytotoxin-associated gene A and vacuolating cytotoxin A (VacA) to initiate inflammatory and immune responses that promote cancer[48,49]. Mechanistically, cytotoxin-associated gene A destabilizes intercellular junctions of gastric epithelial cells and activates pro-tumoural proliferation pathways such as the MAPK pathway[48] or it can interfere with tumour-suppressor genes through epigenetic modulation[50]. VacA is internalized by gastric epithelial cells where it induces multiple effects, including vacuole formation, mitochondrial membrane alteration, stimulation of apoptosis and disruption of cell signalling pathways (for a review, see ref. 51). Interestingly, although *H. pylori* initiates gastric carcinogenesis, it has been shown to be significantly depleted in advanced GC, attributable to the atrophic niche, an environment characterized by the progressive loss of specialized glandular tissue, reduced mucus production and impaired gastric acid secretion, which promotes the survival of non-*H. pylori* species in the stomach[43]. Deep sequencing of amplified 16S rDNA from biopsy samples from two different populations in Colombia with similar *H. pylori* prevalence, found that *Leptotrichia* and *Veillonella* were significantly more abundant in one of the two populations and that these individuals have a 25-fold higher risk of GC compared to the other population[52]. This leads to the possibility that diverse bacteria residing within the gastrointestinal tract may interact synergistically with *H. pylori*, thereby contributing to the pathogenesis of GC.

Intestinal microbiota alteration has been highlighted in GC cases[53], and it has been shown that during gastric carcinogenesis, the species richness of gut bacteria increases while the species diversity decreases, with the most significant changes detected in the precancerous lesion of the GC group[54]. For instance, *Propionibacterium acnes*, enriched in *H. pylori*-negative GC tissues and correlated with advanced stages, promotes tumour progression by inducing M2 macrophage polarization via the TLR4–PI3K–Akt pathway[55]. Other intestinal species such as *F. nucleatum*, well known in CRC tumorigenesis, modulate the tumour microenvironment to support cancer progression[56]. As shown in germ-free mouse models, *Streptococcus anginosus* disrupts the gastric barrier, enhances cell proliferation and inhibits apoptosis, driving GC development[57]. Furthermore, following eradication of *H. pylori*, studies have shown marked changes in the microbial composition of the stomach that may be associated with gastric atrophy and intestinal metaplasia, suggesting that eradication of *H. pylori* alone may not be sufficient to prevent GC[58].

Not only gastric and intestinal, but also oral, deviations of the bacterial composition have a role in gastric carcinogenesis. Oral microbiota, including the salivary microbiota, and GC have also been described[59,60] opening the possibility to search for non-invasive biomarkers of GC[60].

In summary, although *H. pylori* is the primary bacterium associated with GC, perturbations in the gastrointestinal microbiota also contribute extensively to disease progression.

Liver cancer. The intestinal microbiota also has a role in the advancement of hepatocellular carcinoma (HCC; see ref. 61 for a review). Most HCC cases develop in people with hepatic cirrhosis, a condition that is often associated with substantial changes in gut microbiota composition. For instance, increases in *Bacteroides*[62], Ruminococcaceae[62] and Actinobacteria[63], as well as *E. coli*[64], have been observed in the faecal microbiota of individuals with liver cancer and/or cirrhosis. Although no specific gut microbiota signature has been definitively linked to HCC, these findings suggest that the gut microbiota is altered during the progression of liver diseases[65].

The gut and the liver are interconnected through bidirectional communication pathways involving the biliary tract, portal vein and systemic circulation. Research summarized in literature reviews suggests that impaired gut barrier permeability and deviations of the gut microbiota are important components in the development and progression of HCC[66]. Disruption of the gut barrier function allows for the translocation of bacteria, bacterial metabolites, ligands and endotoxins from the intestine to the liver, which can induce carcinogenesis. Numerous processes have been highlighted, such as inflammation, fibrogenesis, hepatocyte injury, regeneration and immunity[67]. In a clinical study searching for faecal biomarkers for early HCC, SCFA-producing bacteria were reduced in individuals with early HCC compared to

healthy individuals[63]. This finding underscores again the pivotal role of gut microorganisms in cancer development and progression, with immunity and metabolism as critical mediators. A compelling example is the decrease in *Bacteroides thetaiotaomicron* in faecal samples, a SCFA-producing species that has been significantly associated with HCC recurrence after surgery[68]. *B. thetaiotaomicron*-derived acetate enhances the immune microenvironment by promoting the polarization of tumour-associated macrophages towards an antitumorigenic M1 phenotype. This polarization is crucial as M1 macrophages release cytokines that stimulate cytotoxic $CD8^+$ T cells. Beyond immune modulation, acetate also exerts epigenetic effects on key enzymes involved in fatty acid metabolism, contributing to a metabolic shift that supports antitumour immunity in the context of HCC[68].

Most HCC cases develop in individuals with chronic liver disease, caused by viral infection, non-alcoholic fatty liver disease and alcohol-related fatty liver disease. Gut microbiota analysis revealed distinct differences between viral and non-viral HCC. In non-viral HCC, 16S rRNA sequencing showed more pro-inflammatory bacteria and fewer SCFA-producing bacteria[66], while individuals with viral HCC had more anti-inflammatory species[69]. Alcohol consumption in non-viral HCC correlated with specific bacteria and increased gut permeability, with markers of microbial translocation and intestinal damage elevated[66]. These findings suggest gut microbiota signatures could help in distinguishing HCC subtypes.

Notably, by modulating bile acid (BA) metabolism, deviations of the gut microbiota can promote the development of liver cancer. Primarily synthesized in the liver, they do so through multiple mechanisms, including the induction of hepatocyte apoptosis, promotion of chronic inflammation and modulation of immune responses (see ref. 70 for a review). This topic will be discussed further later in this Review.

Pancreatic cancer. Risk factors such as obesity, diabetes and chronic inflammation are strongly associated with pancreatic ductal adenocarcinoma (PDAC), and each of these is markedly influenced by the gut microbiota. For instance, an imbalanced microbiome can contribute to low-grade systemic inflammation, insulin resistance and altered lipid metabolism, all of which may increase susceptibility to pancreatic cancer[71]. Besides the well-described role of the oral microbiome, including *Porphyromonas gingivalis*[61], several studies also highlighted a link between intestinal bacteria and the development of pancreatic cancer[72]. During PDAC development, substantial alterations in the gut microbiota and a compromised intestinal barrier are commonly observed. This disruption allows certain gut microorganisms to translocate to the pancreas, where they can colonize and create a suppressive microenvironment that facilitates tumour progression. Again, gut microbial diversity was shown to be decreased in 16S rRNA-sequenced stools from individuals with pancreatic cancer and linked with an increase of LPS-producing bacteria and a decrease of butyrate-producing bacteria[61,73]. For example, in a study aimed at finding early biomarkers, it was observed that Proteobacteria (recently renamed as Pseudomonadota) and Firmicutes (renamed as Bacillota) were dominant in the faecal microbiota in individuals at early stages of PDAC development[74,75].

It has been hypothesized that bacteria may influence the progression of PDAC through different mechanisms. First, gut bacteria may reach the pancreatic tissue through pancreatic duct reflux of bile or through the lymphatic or portal circulation. Deviations in the composition of intrapancreatic microbiota have been observed in individuals with pancreatic cancer compared to healthy control individuals but also compared to the gut microbiota[76]. Mechanistically, the tumoural microbiome seems to induce local tumoural immunosuppression and T cell exhaustion through activation of TLRs in monocytic cells[77]. Second, the gut microbiota could be associated with the development of pancreatic cancer through its multiple inherent interactions with the metabolism of BAs. Interestingly, many risk factors for pancreatic cancer such as obesity, chronic pancreatitis, hypertriglyceridaemia and diabetes are also associated with alterations in the composition of BAs and BA reflux into the pancreatic duct[78]. Carcinogenic and beneficial BAs will be detailed in a specific section of this Review.

Deviations of the gut microbiota composition and activity could explain part of the development of chronic pancreatitis and later pancreatic cancer. Several factors have been identified, including higher levels of LPS[73], lower levels of trimethylamine *N*-oxide (TMAO) and chronic local release of pro-inflammatory cytokines and reactive oxygen species[79]. These alterations can lead to intracellular DNA damage, induction of hypoxia signalling and cellular dedifferentiation through the epithelial–mesenchymal transition, all of which have a role in cancer progression[80].

Changes in gut microbiota composition in hormone-dependent cancers. *Breast cancer*. The gut microbiota is actively involved in hormone metabolism, influencing the progression or regression of hormone-dependent tumours. Among these, the association between breast cancer (BC) and the so-called 'estrobolome' is the most extensively studied. The estrobolome refers to the collection of bacterial genes that encode enzymes capable of metabolizing oestrogens and their metabolites, thereby affecting their circulating levels in the body[81,82]. Oestrogens are metabolized in the liver, where they are conjugated and excreted in the bile into the gastrointestinal tract. In the gastrointestinal tract, these conjugated oestrogens can be reabsorbed into the bloodstream as free oestrogens after being deconjugated by certain enzymes. Enzymes such as the bacterial β-glucuronidases and β-glucosidases have a crucial role in this deconjugation process, thereby facilitating the enterohepatic circulation of oestrogens[83]. Moreover, antibiotic use suppresses this bacterial deconjugation, leading to increased faecal excretion of conjugated steroids[84]. After deconjugation, oestrogens regain their active form and can bind to oestrogen receptors, promoting the cell cycle and resulting in increased cell proliferation[85]. An estrobolome enriched in genes encoding enzymes promoting oestrogen metabolite deconjugation reactions results in increased reabsorption of free oestrogens and influences the carcinogenesis of oestrogen-driven tumours. In BC, the enrichment of species presenting β-glucuronidase activity (*Clostridium coccoides*, *Clostridium leptum*, *Blautia* spp.) may contribute to the progression of more severe clinical stages[86].

Moreover, gut microbiota deviation is associated with obesity (see reviews in refs. 2,3,7,71), a major risk and outcome factor for BC, which leads to abnormal sex hormone production in the adipose tissue via the enzyme aromatase and contributes to hormone reabsorption from the gut[87,88].

Studies investigating the gut microbiota in relation to BC have produced contradictory results, and no specific microbial profile has been established as a conclusive biomarker. In postmenopausal women with BC, some studies have reported significantly higher α-diversity and β-diversity compared to healthy control individuals[89]. By contrast, it was found that the faecal microbiota of postmenopausal women with BC is less diverse than that of matched healthy women[90,91], while another study reported no significant differences in richness, diversity or overall composition between these groups[92]. Metagenomic analysis has shown that although the gut microbiota of premenopausal women with BC does not differ significantly in diversity from that of healthy counterparts, the taxonomic composition and functional capacity of the gut microbiota in postmenopausal women with BC are significantly different from those in healthy control individuals[89]. Yet the overall composition of the microbiota does not seem to change significantly between premenopausal and postmenopausal women[93].

Certain bacterial species are known to produce metabolites that influence systemic inflammation and immune responses, which are critical factors in tumorigenesis. For example, oral administration of *Prevotella copri* in either specific pathogen-free mice or germ-free

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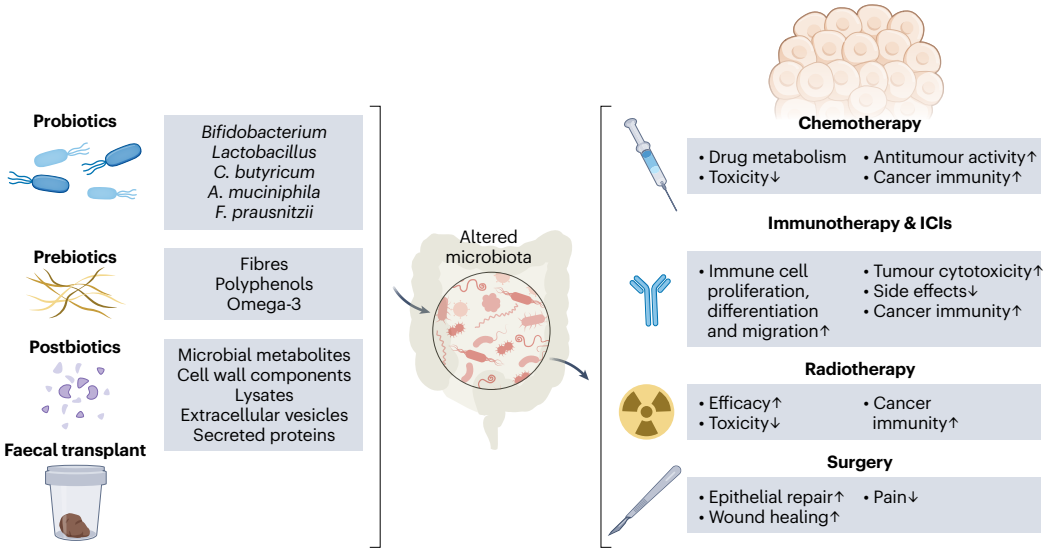

Fig. 2 | Gut microbiome-based therapies against cancer and their impact on treatments. This figure shows how different microbiome-based interventions, such as probiotics (for example, *Bifidobacterium*, *Lactobacillus*, *C. butyricum*, *A. muciniphila* and *F. prausnitzii*), prebiotics (for example, fibres, polyphenols and omega-3), postbiotics (for example, microbial metabolites, cell wall components and extracellular vesicles) and faecal transplants, can alter gut microbiota composition and functionality. These changes can influence the effectiveness of various cancer treatments, including chemotherapy, immunotherapy (such as ICIs), radiotherapy and surgery, by enhancing drug metabolism, reducing toxicity, promoting antitumour immunity, improving immune cell proliferation and migration, reducing side effects and promoting wound healing. Ultimately, these strategies aim to optimize therapeutic outcomes and strengthen anticancer responses.

mice resulted in an extensive depletion of indole-3-pyruvic acid (a putative protective tryptophan-derived metabolite), thereby promoting BC progression by inactivating certain tumour-suppressing pathways. Indole-3-pyruvic acid activates the AMP-activated protein kinase signalling pathway, leading to reduced ATP production and impairing mitochondrial oxidative phosphorylation and glycolysis, thereby limiting the energy available for tumour growth[94]. It also has an epigenetic role in altering DNA methylation processes of genes involved in cancer progression[94].

Depletion of SCFA-producing bacteria such as *F. prausnitzii* may also contribute to BC development by suppressing proliferation through other inflammatory pathways such as interleukin (IL)-6–JAK2–STAT3 (ref. 95), also known to contribute to the epithelial–mesenchymal transition and metastasis development. It is worth noting that *F. prausnitzii* also exerts an antitumour effect in mice by inducing apoptotic activity[95]. The intestinal colonization of some species may also have an impact on the progression of BC metastasis[96,97]. ETBF colonization may establish premetastatic niches in organs such as the liver and lung to enhance metastatic dissemination by upregulating inflammation, remodelling the tumour microenvironment and creating an immunosuppressive microenvironment[98]. In mouse models, inflammation induced by the systemic response to gut microbiota deviation precedes the development of lymph node and lung spreading, which is recapitulated both by direct targeting of gut microorganisms with antibiotics and by FMT of caecal contents from altered microbiota[99].

Prostate cancer. Similar to oestrogens, conjugated androgens can be deconjugated in the intestinal tract by bacterial β-glucuronidase to free androgens for reabsorption into the bloodstream. Serum testosterone levels correlate significantly with the abundance of *Acinetobacter*, *Dorea*, *Ruminococcus* and *Megamonas* in faecal samples[100]. In addition, the gut microbiota has a role in androgen biosynthesis via the conversion of cortisol metabolites derived from bacteria, which enhances proliferation and migration of prostatic cancer cells[101]. In individuals with prostate cancer undergoing androgen deprivation therapy, as well as in castrated mice, certain gut bacteria have been found to produce androgens that enter the systemic circulation[102]. These gut-derived androgens seem to promote prostate cancer growth and contribute to the development of resistance to endocrine therapy. FMT from endocrine-resistant individuals has been shown to induce castration resistance in mice with prostate cancer[102].

Interaction between treatments and microbiota

Interactions between chemotherapy and microbiota. In addition to the role in carcinogenesis, the gut microbiota modulates the response to chemotherapy treatment, including efficacy and toxicity of the drugs. This modulation occurs through several mechanisms such as inducing microbial enzymatic modulation of the chemotherapeutic agents, modulating metabolic pathways and enhancing the immune system. Some bacterial enzymes can convert prodrugs to their active forms or conversely deactivate them, reducing their effectiveness against cancer cells[103]. For example, in the case of gemcitabine, a drug commonly used to treat PDAC, specific bacteria can convert it into its inactive form through a bacterial enzyme called cytidine deaminase, which contributes to drug resistance[103]. Gut microorganisms can also interfere with metabolic pathways linked with chemotherapy efficacy. In addition to its role in the development and progression of CRC, high levels of *F. nucleatum* are known to be associated with 5-fluorouracil resistance in individuals with CRC[104]. *BIRC3* has been found to be the most upregulated gene induced by *F. nucleatum* in CRC cell lines[104]. This gene contributes to the inhibition of apoptosis by inhibiting the caspase cascade, which may contribute to chemoresistance[104]. *F. nucleatum* may also induce drug resistance by inhibiting the ferroptosis pathway[105]. This species can also activate autophagy pathways through targeting microRNAs and TLR4–MYD88 signalling, allowing cancer cells to survive[25]. In PDAC, bacterial metabolites such as tryptophan-derived indole-3-acetic acid (3-IAA) have been associated with enhanced chemotherapeutic efficacy through the modulation of metabolic pathways. As demonstrated by studies using FMT and short-term dietary modulation, it induces the accumulation of reactive oxygen species and downregulation of autophagy, thereby contributing to reduced metabolic fitness and decreased cell survival after chemotherapy[106].

In addition, specific microbial species may enhance the anticancer immune response associated with chemotherapy (Fig. 2).

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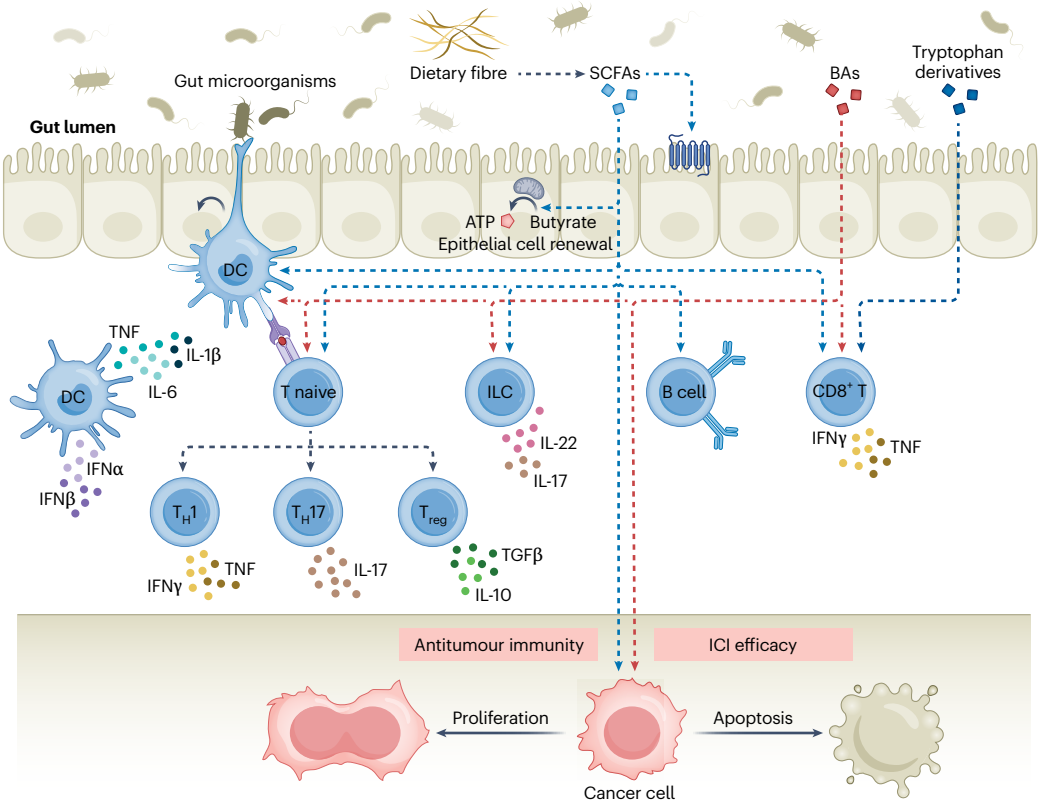

Fig. 3 | Gut microbiota metabolites and immunity. This figure depicts the interactions between the gut microbiota and the immune system, illustrating how dietary components such as fibre influence the production of SCFAs, BAs and tryptophan derivatives. These metabolites modulate various immune cells (for example, DCs, T_H1, T_H17, T_{reg} cells, $CD8^+$ T cells and B cells) and promote antitumour immunity and ICI therapy efficacy by regulating inflammatory cytokines (for example, IFNγ and TNF) and enhancing cancer cell apoptosis.

In antibiotic-treated mouse models, the efficacy of oxaliplatin, a platinum-based chemotherapeutic agent in treatment of CRC, has been shown to be enhanced by metabolites such as butyrate[107]. Butyrate, which was more abundant in individuals who responded to the drugs, modulates the function of $CD8^+$ T cells in the tumour microenvironment, which promotes antitumoural immune response. Cyclophosphamide, an alkylating agent, facilitates the translocation of *Enterococcus hirae* and *Barnesiella intestinihominis* to the lymph nodes where they induce a T helper 17 (T_H17) cell immune response, crucial for controlling cancer growth[108] (Fig. 3). Reduced microbial diversity, particularly a decrease in butyrate and indole-3-propionic acid-producing species, has been associated with poor treatment outcomes. In individuals with BC who were treated with neoadjuvant chemotherapy, species such as *Coprococcus*, *Dorea* and *Ruminococcus* were reduced in non-responders and correlated with levels of peripheral and tumour-infiltrating $CD4^+$ T cells[109] (Fig. 2).

Although chemotherapy is designed to target cancer cells, it can also affect normal cells, leading to a variety of side effects, such as diarrhoea, mucositis, haematological suppression, hair loss, and nausea/vomiting. Research has highlighted the role of the gut microbiota in mediating the toxicity of chemotherapeutic agents. For example, gut microorganisms involved in enterohepatic circulation can prolong the effect of drugs by increasing their intestinal reabsorption, thus modulating systemic exposure to the drugs and their metabolites. This has been demonstrated with the chemotherapeutic agent irinotecan, whose major toxicity, diarrhoea, is increased by the action of the intestinal microbial enzyme β-glucuronidase[110]. Commensal species affected by chemotherapy may contribute to weight gain and neurological side effects during BC treatment[111]. Analysis of pre-chemotherapy stool samples using shotgun metagenomics revealed that high α-diversity is linked to a reduced risk of haematological toxicities, such as neutropenia[112]. Additionally, specific microbial species were associated with either a reduced or an increased risk of severe neutropenia, as well as severe nausea and vomiting[112]. The role of the microbiota in chemotherapy treatment outcomes is supported by studies indicating that antibiotic use is associated with poorer responses to chemotherapy, including reduced disease-free survival and overall survival[113,114]. However, this might not be limited to the use of antibiotics and could also be linked to other possible drugs because many non-antibiotic drugs have been shown to change microbiota composition[115]. By comparing pre-chemotherapy and post-chemotherapy stool samples, an expansion of commensals associated with favourable or unfavourable prognosis is also observed[111].

Interactions between radiotherapy and microbiota. Radiotherapy can interact with the gut microbiota in a bidirectional manner: radiation therapy can alter the microbiome by increasing deviations of the gut microbiota, but some bacteria can also affect the effects of radiation therapy[72,116] (Fig. 2). The mouse intestinal microbiota was shown to be modified after radiotherapy, where irradiation treatment increased the level of *Alistipes* in the large intestine and increased the level of *Corynebacterium* in the small intestine[117]. A recent study also showed that radiation altered the microbial distribution within each of the main phyla in a dose-dependent and tissue-dependent manner, with an increased abundance of bacteria from Bacteroidetes (Bacteroidota) in the colon and faeces[118]. It was also shown that FMT protects against radiation-induced toxicity in mice[119].

A clinical study of patients with gynaecological cancer has shown that pelvic radiotherapy induced a reduction in the numbers of species-level taxa constituting the gut microbiota, with a 10% reduction

in Firmicutes (Bacillota) and a 3% increase in *Fusobacterium*[120]. A large clinical study addressed the association of the gut microbiota with acute and late radiation-induced enteropathy[121]. In the acute cohort, the authors observed a trend for higher diversity in patients before the radiotherapy with no self-reported symptoms, but a consistent association between low bacterial diversity and late radiation enteropathy was observed. They also notably associated radiation enteropathy with higher counts of *Clostridium* IV, *Roseburia* and *Phascolarctobacterium*[121]. Of note, intestinal microbiota composition has been shown to be predictive of radiotherapy-induced acute gastrointestinal toxicity in individuals with prostate cancer[122] and those with head and neck squamous cell carcinoma[123]. Bacterial supplementation is considered to mitigate radiation-induced gastrointestinal damage and microbial alteration[124]. This approach is also currently being studied to enhance radiation treatment efficacy[125]. A case study was also recently reported in which FMT reduced chronic radiation enteritis induced by radiotherapy of a cervical tumour[126]. Finally, microbiome profiling in patients with cervical cancer with acute radiation enteritis was able to classify radiation enteritis and severe acute radiation enteritis[127]. These potential therapies targeting the microbiota are further discussed in this Review.

Interactions between surgery and microbiota. The role of the gut microbiota in surgery, particularly gastrointestinal surgery, has been extensively studied. Preoperative deviations of the gut microbiota composition, identified through stool Gram staining, are linked to higher rates of postoperative complications such as infections, anastomotic leakage and other adverse events[128]. Specifically, a high abundance of Lachnospiraceae and Bacteroidaceae, along with lower microbial diversity, is strongly associated with anastomotic leakage[129]. Hajjar et al.[130,131] further demonstrated that preoperative gut microbiota can causally affect the healing of anastomotic leakage in CRC. By using human faeces transplanted in mice, they found elevated levels of mucosal pro-inflammatory cytokines before surgery impaired healing. They identified two bacterial species with opposing effects. *Alistipes onderdonkii* kh33 impaired healing by upregulating pro-inflammatory cytokines, whereas *Parabacteroides goldsteinii* kh35 promoted healing by restoring the gut barrier and reducing inflammation.

It is worth noting that cancer surgery targeting sites other than the intestine can also alter the gut microbiota. For example, studies comparing preoperative and postoperative stool samples from patients with breast[132] and ovarian[133] cancer revealed important shifts in bacterial communities and metabolites. These findings highlight the need for comprehensive postoperative management, including wound care and dietary adjustments. Both long-term and short-term preparations for intestinal surgery impact the gut microbiota. Long-term interventions such as neoadjuvant radiotherapy or chemotherapy, and short-term measures such as fasting, bowel preparation and antibiotic prophylaxis, have considerable roles. Although oral antibiotic bowel preparation has been associated with reduced surgical site infections[134], it also disrupts the gut microbiota, and effective alternatives are still lacking[135].

The gut microbiota is crucial for intestinal wound healing and epithelial repair, partly through immunomodulation and the production of metabolites such as butyrate (Fig. 2). Specific microorganisms, such as *Akkermansia muciniphila*, have been identified as key regulators of intestinal wound healing through reactive oxygen species-dependent mechanisms and formyl peptide receptors[135,136]. In mouse models, preoperative administration of *A. muciniphila* for 2 weeks has been shown to enhance intestinal wound healing by activating TLRs and strengthening the gut barrier[137]. This effect on transcriptomic activation of the TLR pathway was also observed in colonic biopsy samples from healthy humans exposed for 2 h to *A. muciniphila*[137].

Additionally, the gut–brain axis has a role in persistent postoperative pain. Certain bacterial taxa, including *Bifidobacterium longum* and *F. prausnitzii*, are associated with the absence of pain, while others, such as *Megamonas hypermegale* and *Bacteroides pectinophilus*, are linked to persistent pain after BC surgery. Imbalances in the gut microbiota and the production of microbial metabolites may contribute to these outcomes by promoting peripheral sensitization and pro-inflammatory responses[138].

Interactions between targeted therapy and microbiota. Although the association between most types of therapy, including immunotherapy, and the gut microbiome has been extensively studied, less is known about the link between targeted therapies and microbiota. A study on metastatic CRC assessed the association between the gut microbiota and response to targeted therapies including anti-epidermal growth factor (cetuximab) and anti-vascular growth factor (bevacizumab), showing a higher diversity in the gut microbiota of the progressive disease group in comparison with the partial response group. Of note, *F. nucleatum* was about 32 times higher in the progressive disease group than in the partial response group[139]. The gut microbiota was also shown to be involved in response to combined cetuximab and avelumab in patients with metastatic CRC and non-small cell lung cancer (NSCLC)[140], showing that *Agathobacter* and *Blautia* could be potential biomarkers of outcome in those patients. Interestingly, it was shown in a small cohort of patients with NSCLC that chemotherapy altered the gastrointestinal microbiota more than EGFR-targeted tyrosine kinase inhibitors[141].

In BC, the composition of the gut microbiota has been linked to the efficacy of targeted therapies. In a cohort of 14 patients with metastases, faecal microbiota composition was associated with the response to CDK4/CDK6 inhibitors combined with endocrine therapy[142]. In HER2-positive BC, the antitumour effect of trastuzumab, an HER2-targeted therapy, was shown to diminish following antibiotic use in patients or after FMT from antibiotic-treated donors in mice[143]. Moreover, when FMT from responders and non-responders to neoadjuvant trastuzumab was administered to mice with HER2-positive BC, the drug response observed in patients was successfully replicated, highlighting the importance of the gut microbiome[143]. Additionally, an analysis of the gut microbiota identified a signature associated with a reduced risk of gastrointestinal toxicity induced by neratinib, a tyrosine kinase inhibitor used to treat HER2-positive BC[144]. With respect to melanoma treatment, little is known about MAPK-targeted therapy (BRAF/MEK inhibitors) with a lack of association studies in patients with melanoma[145]. However, patients with CRC who have *BRAF* mutations were characterized by an abundance of *Fusobacterium*, *Prevotella enoeca*, *Prevotella dentalis*, *Hungateiclostridium saccincola*, *Sutterella megalosphaeroides*, *Stenotrophomonas maltophilia* and *Victivallales* bacterium[145].

Microbiota-driven immune regulation and its impact on cancer immunotherapy

The gut microbiota has a fundamental role in regulating immune homeostasis and influencing host responses to cancer therapies. Emerging evidence highlights its capacity to modulate both innate and adaptive immunity, shaping the tumour microenvironment and affecting the efficacy of immune-based treatments. This section explores the principles of cancer immunotherapy and how microbiota–immune interactions contribute to therapeutic responses.

Immunotherapy: general principles

The involvement of the immune system in monitoring and controlling tumour growth is now widely recognized, with tumour development and progression often associated with an impaired or exhausted antitumour immune response. This compromised state is characterized by the upregulation of inhibitory molecules, such as immune checkpoints or immunosuppressive cytokines and chemokines, thereby limiting the effectiveness of the antitumour activity[146]. Immune checkpoints are regulatory pathways in the immune system that control

immune responses to prevent overactivation and ensure self-tolerance. Checkpoint molecules negatively regulate T lymphocyte activation to fine-tune the immune system. PD-1 and cytotoxic T lymphocyte antigen 4 (CTLA-4), the two main immune checkpoint proteins, function as co-inhibitory receptors found on T cells downregulating their activation and preventing excessive immune responses[147]. Although this mechanism is crucial for maintaining immune homeostasis and preventing autoimmunity, tumour cells exploit these pathways to evade immune surveillance, leading to tumour tolerance and T cell exhaustion. Immune checkpoint inhibitors (ICIs), such as anti-CTLA-4, anti-PD-1 and anti-PD-L1 (a ligand for PD-1) antibodies, have revolutionized cancer therapy by blocking these co-inhibitory signals, thereby reactivating T cells and restoring their ability to target and destroy tumour cells. The identification of immune checkpoint proteins such as PD-1/PD-L1 and CTLA-4 has marked a major advancement in cancer immunotherapy, offering new therapeutic avenues for enhancing anti-tumour immune responses.

Microbiota–immune interactions

Understanding the intricate mechanisms by which microorganisms influence the immune system is becoming increasingly vital for evaluating and optimizing their impacts in cancer therapy, particularly immunotherapy. The gut microbiota triggers anticancer immune responses through various pathways (Fig. 3), with key mechanisms including:

1. Stimulating T cell responses. Gut microorganisms can stimulate T cell responses against microbial antigens, which may either directly enhance tumour-specific immune responses or lead to cross-reactivity with tumour-specific antigens. This cross-reactivity can contribute to the recognition and destruction of cancer cells by the immune system.
2. Activating pattern recognition receptors. Gut microorganisms activate pattern recognition receptors such as TLRs and nucleotide-binding oligomerization domain-like receptors, which can lead to either pro-immune or anti-inflammatory outcomes. The activation of these receptors triggers a cascade of signalling events that can enhance antitumour immunity or, conversely, dampen inflammatory responses to maintain immune homeostasis.
3. Producing systemic metabolites. Microbial metabolites, such as SCFAs, tryptophan derivatives and BAs, have systemic effects on the host, including modulation of the immune response. These metabolites can influence the function of various immune cells, including T cells, DCs and macrophages, thereby shaping the overall immune landscape within the tumour microenvironment. These metabolites will be detailed in the next part of this Review (Fig. 3).

In line with the different response rates to ICIs, recent studies increasingly highlight the gut microbiota and its metabolites as critical modulators of ICI efficacy by modulating both innate and adaptive immune responses, including the regulation of T cell function and cytokine production. The gut microbiota is also essential for maintaining intestinal barrier integrity and modulating mucosal immunity, thereby protecting the host from pathogenic infections and preventing overexposure to commensal bacteria[7,71,148]. This protective role is crucial not only for gut health but also for systemic immune regulation, as breaches in the gut barrier can lead to systemic inflammation and immune dysregulation, which may affect cancer progression and treatment outcomes. Moreover, the gut microbiota influences several key physiological processes, including metabolism, inflammation, haematopoiesis and immunity. These processes are fundamental to the development and functionality of the immune system, highlighting the multifaceted role of the microbiota in health and disease. The interplay between the gut microbiota and the immune system is complex and involves the maintenance of immune balance and the formation of the intestinal T cell repertoire (Fig. 3). It also serves as a major source of specific antigens that activate T cells. These interactions promote the development of adaptive immune responses targeting specific gut bacterial antigens[149].

Microbiota metabolites potentially contributing to immune modulation

Bacteria and their metabolites have a substantial role in the regulation of the immune system, influencing both inflammatory processes and immune suppression. In the context of cancer, bacterial metabolites can promote inflammation or contribute to an immunosuppressive tumour microenvironment. Specifically, SCFAs, BAs and tryptophan-derived metabolites have been identified as key contributors to immune modulation in the tumour microenvironment[150–152] (Fig. 3).

SCFAs. SCFAs such as acetate, propionate and butyrate are microbial metabolites generated through the fermentation of non-digestible carbohydrates such as dietary fibres[153]. They have a crucial role in maintaining gut health and influencing various physiological processes, both locally in the gut and systemically.

SCFAs have been the focus of numerous studies demonstrating their ability to modulate the immune system, and numerous studies have linked SCFA levels with cancer prevention[154], particularly CRC, with reductions in SCFA-producing bacteria noted in individuals with CRC[155]. Interestingly, this trend has also been observed in other types of cancer[156,157]. Because SCFA levels are measured after cancer development, it is difficult to determine whether their reduction is a cause or consequence of cancer. However, numerous in vitro studies support an active role for SCFAs, especially butyrate, in tumour suppression[158].

SCFAs exert their effect through G-protein-coupled receptors, such as GPR43, GPR41 and GPR109A, which have key roles in immune response and tumour suppression[159] (Fig. 3). Decreased expression of these receptors has been observed in several cancers, including CRC[160], and restoring their function has led to increased sensitivity to chemotherapy and the induction of apoptotic pathways. For example, GPR109A, when re-expressed in cancer cells and treated with butyrate, promotes the expression of pro-apoptotic genes while downregulating anti-apoptotic proteins such as Bcl-2 (ref. 161).

SCFAs also act by inhibiting histone deacetylase (HDAC) activity, which has been widely recognized for its antitumour effects, as it prevents the removal of acetyl groups from histones, thereby maintaining an open chromatin structure and promoting the transcription of genes involved in cell cycle arrest and apoptosis[162]. As HDAC inhibitors, SCFAs are thought to have important effects on transcriptional regulation and sensitivity and/or resistance to cancer therapeutics[163]. Co-treatments with SCFAs, like the combination of propionate with cisplatin, have shown enhanced therapeutic efficacy, as demonstrated by the induction of more apoptosis and reduced HDAC activity in cancer cells[164].

Beyond chemotherapy, SCFAs are emerging as potential boosters for cancer immunotherapy. Through their ability to modulate a wide variety of immune cells, including type 3 innate lymphoid cells (ILC3s), neutrophils, dendritic cells (DCs), macrophages, B cells, regulatory T (T_{reg}) cells, T helper 1 (T_H1) cells and $CD4^+$ and $CD8^+$ T cells (Fig. 3). This immune modulation is achieved via the interactions of SCFAs with G-protein-coupled receptors and HDAC inhibition, which enhance the production and release of anti-inflammatory cytokines, such as IL-10, or pro-inflammatory cytokines, such as IL-1β, influencing inflammatory processes and tumorigenesis[165,166]. One of the primary immune effects of SCFAs is the promotion of T_{reg} cell differentiation, which occurs through HDAC inhibition and the activation of $CD103^+$ DCs (Fig. 3), mediated by GPR43 and GPR109A receptors. These DCs are essential for T_{reg} cell induction in the gut, contributing to immune tolerance and the regulation of the antitumour immune response[167–169]. SCFAs also promote the production of IL-22, a critical cytokine for intestinal immunity, through $CD4^+$ T cells and innate lymphoid cells (ILCs)[170] (Fig. 3). This

effect is mediated via GPR41 and HDAC inhibition, which upregulates IL-22 by enhancing the expression of key transcription factors such as the aryl hydrocarbon receptor (AhR) and hypoxia-inducible factor 1α. Hypoxia-inducible factor 1α directly binds to the IL-22 promoter, and SCFAs enhance this binding through histone modification. While IL-22 is essential for maintaining intestinal barrier function, under certain conditions it can also contribute to cancer spread, by enhancing the metastatic colonization potential and promoting immune evasion[171].

Among the SCFAs, butyrate has been particularly studied in the context of CRC, where it serves as a key energy source for colonocytes, having a crucial role in the renewal of epithelial cells and preserving barrier integrity. This barrier function is essential for maintaining intestinal balance and reducing systemic inflammation by preventing inadequate exposure to microbial products[61,166]. Butyrate also acts through mechanisms such as epigenetic regulation and modulation of signalling pathways. One notable phenomenon, the 'butyrate paradox', refers to butyrate's selective anticancer effects on malignant colon cells while promoting normal colonocyte health[172]. The butyrate paradox is primarily attributed to the metabolic differences between cancerous and non-cancerous cells. Cancer cells, relying on glucose through the Warburg effect[172], accumulate butyrate, which then acts as a HDAC inhibitor. Butyrate's epigenetic impact extends to regulating microRNAs, crucial in cancer progression. For instance, it downregulates oncogenic miR-17-92a, leading to increased expression of tumour-suppressor genes such as *PTEN* and p21 protein, which promote cell cycle arrest and apoptosis[173].

Acetate, another SCFA, has also been extensively reviewed for its role in cancer metabolism[174] as it is closely linked to the acetyl-CoA synthetase short-chain family member 2 (ACSS2) conferring potential metabolic benefits to cancer cells[152]. However, acetate generated by *Bifidobacterium bifidum* and other species enhances cytokine production and cytotoxic activity in $CD8^+$ T cells[175]. Acetate generated by *Lactobacillus reuteri* suppresses IL-17A production by ILC3s in the liver. Additionally, treatment with acetate markedly enhanced the effectiveness of anti-PD-1 therapy in mice[176]. Recently, a study showed that acetate-derived acetyl-CoA triggers the acetylation of c-Myc thereby enhancing the transcription of genes for PD-L1, glycolytic enzymes, monocarboxylate transporter 1 and cell cycle accelerators[177]. Dietary acetate supplementation fosters tumour growth and reduces $CD8^+$ T cell infiltration, while blocking acetate uptake has been shown to impede immune evasion, thereby improving the efficacy of anti-PD-1 therapy[177].

Adenosine, inosine and tryptophan metabolites. Tryptophan is an essential amino acid that cannot be synthesized de novo by human cells and must be obtained through dietary intake. It is the largest of the 20 common amino acids by molecular weight and it is a precursor for several metabolic compounds necessary for the body's growth and development[178]. Most of the digested tryptophan is absorbed in the intestine and is metabolized by the host's cells via three distinct pathways: the kynurenine, serotonin and indole pathways[179]. However, a fraction remains in the intestinal lumen, where it is absorbed by the bacteria that use it for their own growth and simultaneously produce a myriad of bioactive derivatives, primarily utilizing the kynurenine and indole pathways.

Many microorganisms are listed in the literature as being able to metabolize tryptophan[180], leading to the production of tryptamine, indole (including indole-3-carboxylic acid), and various other metabolites such as indole-3-aldehyde (I3A), indole-3-propionic acid and 3-IAA. Several of these tryptophan derivatives act as ligands for AhR and are associated with conditions such as inflammatory bowel disease, neurological disorders and cancers[181]. For example, I3A has been shown to promote interferon gamma (IFNγ) production by directly targeting $CD8^+$ T cells through AhR signalling. It has also been shown that I3A enhances the efficacy of ICIs[182] (Fig. 3). Similarly, a tryptophan-rich diet can facilitate the effectiveness of ICIs with the antitumour effects depending on AhR activity on $CD8^+$ T cells[182]. Therefore, I3A triggers AhR signalling in $CD8^+$ T cells, elevates cytokine production and improves antitumour immune response. This suggests that the administration of I3A may amplify the efficacy of anti-PD-L1 immunotherapy[182] (Fig. 3). Indole-3-propionic acid has been demonstrated to boost the effectiveness of αPD-1 immunotherapy, which relies on $CD8^+$ T cells[183]. Finally, indole-3-carboxylic acid treatment has shown promise in improving the effectiveness of anti-PD-1 immunotherapy[184], further highlighting the potential of tryptophan metabolites in enhancing cancer treatment outcomes.

Adenosine is generated through hydrolysis of ATP. In the tumour microenvironment, particularly under hypoxic conditions, ATP breakdown is increased, and adenosine levels are elevated, contributing to immune escape by tumours[185]. Adenosine exerts its immunosuppressive effects by binding to G-protein-coupled receptors, mainly via A2aR and A2bR[186]. A2aR is expressed in most immune cells, while A2bR is mainly expressed by macrophages and DCs[187]. The mechanisms of immunosuppression by adenosine involve not only direct effects on antitumour effector cells but also indirect effects on antigen-presenting cells and immunoregulatory cells such as T_{reg} cells[185]. Using A2A receptor (A2AR) antagonists or knockout mice, researchers have been able to demonstrate that blocking the adenosine signalling enhances immunity and improves the outcomes of T cell-based immunotherapies[188].

On the other hand, inosine, a purine nucleoside derived from the breakdown (deamination) of adenosine, has recently gained attention for its immunomodulatory properties[189]. Inosine is produced not only endogenously but also by certain gut microorganisms[190] and can influence immune responses through multiple mechanisms[191]. One of its key actions is its interaction with adenosine receptors, including A2AR[192], which is highly expressed on various immune cells, including T cells[189]. While adenosine produces mainly cyclic AMP-biased signalling at the A2AR, inosine-mediated A2AR activation leads mainly extracellular signal-regulated kinase-1 and extracellular signal-regulated kinase-2 phosphorylation, suggesting pharmacological differences between the two agonists[193]. Inosine, but not its metabolic precursor adenosine, also has the capacity for replacing glucose to support growth and cytotoxic function of T effector cells in vitro[194]. Mager et al. showed that inosine produced by *Bifidobacterium pseudolongum* in the gut was able to reach A2A receptors of T lymphocytes infiltrating tumours and improved the efficacy of anticancer immunotherapy in mice[189,195], further supporting the notion that the gut microbiota has a role in the prognosis of tumour development.

BAs. BAs are synthesized in the liver from cholesterol and have a crucial role beyond their traditional function in lipid digestion and absorption. They are important modulators of the immune system and cancer progression. After their production, primary BAs such as cholic acid and chenodeoxycholic acid are secreted into the intestine. Then they undergo extensive transformation by gut microbiota into secondary BAs, such as deoxycholic acid (DCA) and lithocholic acid (LCA)[196]. These secondary BAs possess distinct biological activities that influence various physiological processes, including immune regulation[197].

Similarly to SCFAs, BAs have complex roles in inflammation and cancer, involving both tumour-promoting and tumour-suppressing activities. Although some BAs have protective roles, certain secondary BAs are implicated in carcinogenesis through mechanisms such as DNA damage, activation of the Wnt–β-catenin signalling pathway, and stimulation of cyclooxygenase-2 activity.

BAs exert their effects primarily through two key receptors: the farnesoid X receptor (FXR) and the G-protein-coupled bile acid receptor 1 (TGR5). FXR is a nuclear receptor found mainly in the liver and intestines. It regulates genes involved in BA synthesis, lipid metabolism and glucose homeostasis[196]. Importantly, FXR also modulates immune responses by influencing the production of inflammatory

cytokines and the activation of immune cells, often leading to an anti-inflammatory environment. This anti-inflammatory role may affect cancer progression and the response to therapies, including ICIs. Recent studies indicate that certain BAs can accelerate CRC progression by promoting DNA damage and reducing FXR signalling in colon cancer cells. This reduction in FXR signalling compromises the epithelial barrier's integrity and enhances Wnt–β-catenin signalling, which is crucial for tumour development. These findings suggest that targeting primary and secondary BAs could be a viable strategy for CRC prevention. Additionally, excessive BAs have been associated with HCC, highlighting their role in liver cancer progression.

TGR5 is a membrane receptor found in various tissues and is highly expressed in immune cells[198]. It is involved in metabolic expenditure, gut motility, glucose homeostasis, BA homeostasis and inflammatory response[199]. TGR5 responds to BAs by activating the adenylyl cyclase–cyclic AMP–protein kinase A signalling pathway and thereby regulate immune cell function[200]. Activation of TGR5 in macrophages, DCs and T cells has been shown to promote an anti-inflammatory state by regulating cell migration and polarization, as well as the production of inflammatory mediators[201,202] (Fig. 3). This receptor also influences the balance between T_{reg} cells and effector T cells, which is crucial for maintaining immune homeostasis[203] and could potentially affect the success of ICIs in cancer treatment.

Despite their carcinogenic potential, secondary BAs such as LCA also exhibit anticancer activity through various molecular mechanisms. For example, LCA can inhibit the proliferation of prostate cancer cells by inducing apoptosis via caspase-3, caspase-8 and caspase-9 pathways[204]. Additionally, LCA has been shown to stimulate antitumour immune responses and reduce BC cell proliferation by 10–20%[205]. These effects were attributed in part to activation of TGR5 by LCA.

Ursodeoxycholic acid (UDCA), a hydrophilic BA, has been investigated for its potential chemopreventive properties against various cancers, particularly CRC[206]. Two decades ago, UDCA was tested in humans. UDCA treatment increased the proportion of UDCA relative to DCA in the aqueous phase of stool, with significant dose–response effects observed at 300 mg and 600 mg per day and no serious adverse events occurred[207]. This finding suggests that UDCA has the potential to enhance BA hydrophilicity and possibly reduce colon carcinogenesis risk[207]. Its mechanisms of action include reducing the concentration of hydrophobic, potentially carcinogenic BAs such as DCA in the colon, thereby decreasing mucosal exposure to these harmful agents[208]. Additionally, UDCA may inhibit cell proliferation and induce apoptosis in cancer cells[209], suggesting that UDCA may have a role in cancer prevention by targeting multiple pathways involved in tumour development and progression. In conclusion, the dual roles of BAs (both promoting and inhibiting cancer) underscore the complexity of their function in cancer biology and suggest potential therapeutic targets.

Moreover, BAs are active at the interface of the host immune system with the intestinal microbiota, determining its complicated role in metabolism and immune response. Alterations in BA metabolism and signalling impact gut homeostasis and induce gut microbial changes. This disruption in gut microbiota not only influences local immune responses but also has systemic effects, potentially affecting the efficacy of cancer therapies such as ICIs. Therefore, modulating BA pathways through diet, pharmaceuticals or microbiota-targeted therapies represents a promising avenue for developing new cancer treatments.

The impact of other bacterial metabolites. Beyond SCFAs, tryptophan metabolites, BAs and inosine, several other bacterial metabolites have been implicated in modulating immune responses and influencing cancer progression, with emerging evidence highlighting their potential role in cancer development and signalling[210].

For example, reuterin is a by-product of glycerol fermentation by *L. reuteri* and is a potent antimicrobial compound[211]. Beyond its role in shaping the gut microbiota, reuterin has been suggested to influence immune cell function indirectly by modulating the composition of gut bacteria, which in turn affects systemic immune responses. Alterations in the microbiome, driven by reuterin production, could enhance the responsiveness of immune cells to immunotherapy, particularly by promoting a favourable immune environment that supports the proliferation of effector T cells and diminishes T_{reg} cell activity[212]. In addition, administration of *L. reuteri* can mitigate the autoimmunity induced by ICIs in mice[213].

Cyclic di-AMP, a bacterial second messenger produced by Gram-positive bacteria, is involved in many bacterial functions such as metabolism, maintenance of osmotic pressure, response to DNA damage and acid stress resistance[214]. In eukaryotic cells, cyclic di-AMP serves as a signalling molecule that is recognized by several receptors and triggers an immune response in the host cells[215]. These pattern recognition receptors include the stimulator of interferon genes (STING) and DDX41 (DEAD-box helicase 41), leading to the production of type I interferons and other pro-inflammatory cytokines[215]. In preclinical models, cyclic di-AMP has been shown to potentiate the effects of ICIs by amplifying the immune system's ability to detect and attack tumour cells. Its ability to act as an immune adjuvant makes it a candidate for combination therapies in cancer treatment[216].

TMAO is a metabolite produced by gut bacteria through the metabolism of dietary choline and has traditionally been studied in the context of cardiovascular disease[217], but recent findings indicate its role in modulating immune responses relevant to different types of cancer[79,218]. A study found that TMAO has antitumour effects in PDAC by modulating immune responses in the tumour microenvironment. TMAO boosts the activation of immune cells such as tumour-associated macrophages and $CD8^+$ T cells, slowing tumour growth. Combining TMAO with ICIs enhanced the effectiveness of the treatment. However, as chronic exposure to TMAO increases risk of cardiovascular disease, chronic inflammatory malignancies, renal failure and diabetes, therapeutic interventions might require cautious strategies[219].

Polyamines, such as putrescine, spermidine and spermine, are small molecules produced by both the host and gut bacteria that are crucial for cell function and replication. Cancer cells need consistently high levels of intracellular polyamines to support ongoing proliferation and so their dysregulated metabolism is common in cancers[220]. Targeting polyamine metabolism is therefore a logical strategy that depletes polyamines or inhibits their synthesis and has shown promise in restoring immune function and enhancing the efficacy of cancer immunotherapies, particularly ICIs[221].

Finally, bacterial cell wall components, such as peptidoglycans and LPS, are recognized by TLRs on immune cells, triggering immune activation. Recent studies, including data on PDAC, have identified the presence of LPS in tumour tissue, which is probably due to gut microbiome translocation or intestinal barrier disruption[222]. TLR4-deficient mice show pro-tumour effects, suggesting that loss of TLR4 signalling impairs antitumour immunity[223]. However, high TLR4 expression in tumours correlates with poor prognosis[224], possibly due to its association with PD-L1 expression[225]. While LPS monotherapy had limited efficacy in reducing PDAC tumour growth, it significantly increased tumour-infiltrating lymphocytes, indicating its potential as an immunotherapy adjuvant. LPS-binding protein helps in the formation of the LPS–TLR4 complex and correlates better with PD-L1 expression than LPS itself, reflecting long-term inflammation. High serum LPS activity in PDAC is associated with both immune activation and increased PD-L1-mediated immunosuppression, suggesting LPS as a potential predictor of PD-L1 blockade therapy response[225]. LPS has been safely used as a vaccine adjuvant in melanoma[226] and could enhance PD-1/PD-L1 immunotherapy efficacy in other types of cancer.

Understanding the diverse roles of these bacterial metabolites in modulating the immune system is crucial for developing novel approaches to enhance cancer immunotherapy. As research advances,

the manipulation of gut microbiota and their metabolites holds great potential for optimizing immune responses, particularly in combination with existing cancer treatments such as ICIs.

Microbiota metabolites potentially contributing to discrepancies in immune modulation and ICI efficacy

The recent opposing findings on *F. nucleatum* in ICI therapy for CRC highlight the complexity of immunomodulation in cancer treatment. Two studies offer contrasting perspectives on how *F. nucleatum* influences the efficacy of anti-PD-1 therapy, sparking a debate on its precise role in the tumour microenvironment. The first study[227] demonstrates that *F. nucleatum* sensitizes microsatellite stable (MSS) CRC to anti-PD-1 therapy. The researchers show that *F. nucleatum* produces butyric acid, which inhibits HDAC3 and HDAC8 in $CD8^+$ T cells. This inhibition increases histone acetylation at the *Tbx21* promoter, enhancing transcription of TBX21, a transcription factor that represses PD-1 expression. The result is reduced T cell exhaustion and improved effector function. Supporting this mechanism, FMT from patients with *F. nucleatum*-high MSS CRC to germ-free mice reproduced the sensitization to anti-PD-1 therapy[227]. These findings suggest that high intratumoural *F. nucleatum* levels may serve as a biomarker of improved immunotherapy response in MSS CRC. Conversely, the second study[228] associates *F. nucleatum* abundance with resistance to anti-PD-1 therapy in metastatic CRC. *F. nucleatum*-derived succinic acid was found to suppress the cGAS–STING–IFN-β signalling pathway, limiting $CD8^+$ T cell trafficking into the tumour microenvironment and thereby reducing antitumour immunity. FMT from non-responders with high *F. nucleatum* levels to mice replicated resistance to anti-PD-1 therapy. Notably, treating mice with metronidazole, an antibiotic targeting *F. nucleatum*, decreased succinic acid levels and restored sensitivity to immunotherapy. These findings position *F. nucleatum* as a potential contributor to immunotherapy resistance through its metabolite-mediated suppression of the immune response. The contrasting roles of butyrate and succinate underscore the metabolic complexity of *F. nucleatum*. The conditions driving the production of these metabolites, such as the specific *F. nucleatum* strains or tumour microenvironment characteristics, remain unclear. In one study examining patients with MSS CRC, *F. nucleatum* abundance predicted better therapy outcomes; while in the second study examining patients with metastatic CRC, high *F. nucleatum* levels were associated with resistance. This suggests that the role of *F. nucleatum* may vary depending on tumour stage or immune context. These studies illustrate the dual role of *F. nucleatum* in CRC, either sensitizing tumours to or inducing resistance against anti-PD-1 therapy, depending on the context. This debate underscores the complexity of microbiota–host interactions and highlights the need for further research. Future efforts should focus on clarifying the conditions under which *F. nucleatum* and eventually specific metabolites enhance or impair therapy, enabling the development of targeted strategies to optimize immunomodulation in CRC.

Microbiota-based therapies against cancers

Given the profound impact of the gut microbiota on immune regulation, inflammation and tumour progression, there is growing interest in harnessing microbial modulation as a therapeutic strategy in cancer treatment. A significant area of current research is focused on modulating the microbiota to create a more favourable immune–microbiota environment that enhances treatment outcomes. Efforts to manipulate the gut microbiota for therapeutic benefit have taken multiple approaches, ranging from broad interventions such as dietary modifications[229,230] and FMT to more targeted strategies involving specific prebiotics[231], probiotics and next-generation beneficial bacteria (Fig. 2). These approaches aim to restore a balanced microbiome, enhance immune activation or mitigate the adverse effects of cancer therapies. While the field is still evolving, early findings highlight the potential of microbiota-based interventions as an adjunct to conventional cancer treatments, paving the way for personalized therapeutic strategies.

Fibres and prebiotics

Nutritional strategies offer an attractive approach for influencing the microbiota due to their probable high safety profile, relatively low cost compared to specific drugs and their non-invasive nature. However, neither the US Food and Drug Administration nor the European Medicines Agency has approved dietary modifications as a stand-alone or adjuvant treatment for cancer. Approvals for cancer therapies require rigorous clinical trials demonstrating efficacy, and no such high-level evidence exists for dietary interventions to 'cure' cancer. Current cancer treatments, such as chemotherapy, radiation, targeted therapy and immunotherapy, are based on high-quality randomized controlled trials and are part of approved treatment protocols. No dietary strategy meets these standards so far. Achieving a high level of evidence will be difficult. For instance, it has been known for several decades that consuming a diet rich in fibre, aligning with the commonly recommended daily intake of 30 grams, is linked to a decreased likelihood of developing various metabolic diseases (for example, diabetes) and inflammatory diseases, along with several types of cancer (including CRC and many others)[232,233] (Fig. 2).

An increased intake of fibre enhanced microbial diversity, and a higher presence of fibre-fermenting microorganisms such as *F. prausnitzii* has been linked to positive responses to ICIs[234–237]. Although diet influences the composition of the microbiota, the interindividual response to a specific dietary change largely depends on the initial composition of their microbiota. For instance, the effects of consuming the same high-fibre diet can vary greatly among different people[238]. Supplementing the diet with targeted prebiotics could serve as a potential method for altering the microbiota. However, this technique might selectively encourage the growth of certain microbial populations instead of increasing the overall microbial diversity. Indeed, a prebiotic is "a substrate that is selectively utilized by host microorganisms conferring a health benefit"[239]. Given the large variety of potential prebiotics tested (that is, non-digestible polysaccharides or oligosaccharides[240], polyphenols[241] and omega-3 (ref. 242)), the variety of possibilities is interesting and might constitute an important area of research in the future (Fig. 2).

The administration of prebiotics before surgery for CRC could influence the host metabolism in the perioperative setting. In a randomized double-blind trial of 139 patients, the administration of fructooligosaccharides, xylooligosaccharides, polydextrose and resistant dextrin for 7 days before CRC surgery led to significant modifications in immunological markers, both in the preoperative and postoperative periods. More specifically, it enhanced the levels of immunoglobulins, as well as transferrin, pointing towards a reduction of circulating inflammatory cytokines[243]. Another randomized study of 73 patients with CRC suggests that supplementation for 7 days with synbiotics (containing both prebiotics and probiotics) before CRC surgery leads to significant reductions in circulation inflammation. This was associated with reduced post-surgical morbidity[244]. This association was also seen in another prospective study of 91 patients[245].

Probiotics and next-generation beneficial bacteria

Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host"[246]. While dietary interventions such as prebiotics aim to feed existing gut microorganisms, probiotics have the unique ability to introduce new microbial species that may not already inhabit the gut. Common probiotic products usually feature single strains of easily culturable microorganisms, such as species from the *Bifidobacterium* and *Lactobacillus* genera that are not butyrate producers. Several studies in mice have shown that oral administration of single probiotic strains from *Bifidobacterium* and *Lactobacillus* might be beneficial to restore the activity of ICIs or to abolish the deleterious effects of antibiotics[247,248] (Fig. 2).

Data converge towards the association between the administration of probiotics and reduced levels of inflammation in patients with cancer. In GC, a meta-analysis showed probiotics were associated with increased levels of albumin, pre-albumin and $CD4^+$ and $CD8^+$ T cells, and reduced levels of IL-6 and tumour necrosis factor (TNF)[249]. In the same tumour type, its added value compared to prebiotics alone is suggested by a prospective study. Patients treated with enteral nutrition after gastrectomy presented significantly less diarrhoea when they received the combined supplementation[250]. In CRC, probiotics are also associated with significantly less inflammation after surgery[251].

This topic is increasingly expanding in research, and specific next-generation beneficial microorganisms have now been identified. Besides the classical probiotics, numerous emerging data are pointing the role of *A. muciniphila* to potentially contribute to the response of ICIs[252–254] and this occurs via numerous mechanisms (see ref. 255 for a review). More precisely, pioneering studies in mice have highlighted the crucial role of the gut microbiota in influencing tumour responses to chemotherapy and immunotherapies targeting PD-L1 or CTLA-4 (refs. 256–258). In 2018, a study investigated whether microbiota changes observed upon antibiotic treatment were linked to malignant disease or could affect primary resistance to PD-1 blockade in tumour-bearing mice and patients with cancer[259]. By profiling the samples from patients with lung and kidney cancers, they found that non-responders to ICIs had low levels of the bacterium *A. muciniphila* (Fig. 2). Remarkably, by using mice and FMT from human donors (responders and non-responders), they found that oral supplementation of *A. muciniphila* to antibiotic-treated mice restored the response to immunotherapy[259]. This study was the catalyst for numerous other investigations into the relationship between ICIs and the gut microbiota[8,235,259–261]. Studies integrating multiple omics approaches to investigate the human gut microbiome are essential for comprehensively understanding its involvement in disease at various functional levels[259,262,263].

Although there is no clear consensus on the specific bacterial taxa linked to ICI response, several clinical studies consistently highlight certain common taxa such as *A. muciniphila*, some species from the *Bacteroides*, *Enterococcus* and *Bifidobacterium* genera but also members of the Ruminococcaceae family, such as *F. prausnitzii* and *Roseburia*[234,237,247,254,259,260,264]. Although the mechanisms may differ across the different genera and species, data strongly support the role of these bacteria in T cell priming and modulation, activation and maturation of DCs. Notably, *A. muciniphila*, *Bifidobacterium* species and *Bacteroides fragilis* have all been found to stimulate DCs to produce IL-12, leading to T_H1 responses, which are crucial for antitumour immunity[234,236,237,247,254,259,260,264,265] (Fig. 2). It is worth noting that a clinical trial is currently ongoing with the aim to test the efficacy of oral administration of *A. muciniphila* to patients with renal cell carcinoma (RCC) or NSCLC under immunotherapy, but whose gut microbiota is deficient in *Akkermansia* (NCT05865730). Nevertheless, the relationship remains complex: in a prospective study of NSCLC treated with ICI or ICI plus chemotherapy, patients with high or no relative abundance of *Akkermansia* demonstrated worse outcomes compared with patients with low abundance of *Akkermansia*. High titres of immune responses against *Akkermansia* were associated with increased gut dysbiosis and elimination of gut bacteria, and higher rates of progressive disease. Thus, carrying high percentiles of *A. muciniphila* may also be deleterious. The abundance of *A. muciniphila* could also have beneficial effects in CRC, by enhancing the efficacy of the chemotherapy regimen FOLFOX (5-fluorouracil, leucovorin and oxaliplatin). In a CRC xenograft model, the benefit of FOLFOX increased with higher levels of the bacteria, while colonization of *A. muciniphila* through bacterial transplantation enhanced the treatment efficacy[266]. In an in vivo and clinical study, as compared with healthy control individuals, its abundance was significantly decreased in patients with inflammatory bowel disease and secondary CRC. Supplementation in mice with colitis reduced the pro-tumorigenic inflammation through expansion and activation of cytotoxic T lymphocytes in the colon and draining lymph nodes[267]. The immune response seems to be mediated by the specific membrane protein Amuc_1100 (refs. 267,268). Independent studies further demonstrate that extracellular vesicles derived from *A. muciniphila* have protective effects in chemically induced colitis[269], and that the protective effects of *A. muciniphila* from CRC development were related to the inhibition of AhR signalling and downstream activation of the Wnt–β-catenin pathways[270].

C. butyricum is another potentially interesting candidate qualified as butyrate-producing bacteria and known for its effects in the gut[246]. *C. butyricum* BM588 has also been widely used in humans as a probiotic. In rodents, several studies have shown improved gut homeostasis and restoration of sensitivity to PD-1 blockade in mice[271]. A study investigated whether this bacterium enhances the efficacy of ICIs and counteracts the reduced response in patients with NSCLC who are administered drugs that induce microbiota deviation (that is, antibiotics or proton pump inhibitors (PPIs)). To test this hypothesis, they conducted a retrospective analysis of 106 patients with stage IV or recurrent metastatic NSCLC, all of whom had been treated consecutively with ICI combinations[272]. The use of *C. butyricum* BM588 was strongly linked to extended overall survival in patients undergoing ICI treatment and markedly enhanced overall survival in those receiving both ICIs and antibiotics or PPIs[272]. Similar data were found in another cohort of patients receiving PPIs[273]. However, these studies were retrospective. So far, only one phase I clinical trial dedicated to advanced RCC tested the addition of *C. butyricum* BM588 to nivolumab and ipilimumab, showing relevant clinical activity of the combined treatment approach[274]. Larger studies are now necessary to confirm these results and to understand the underlying mechanisms affecting the microbiome and immune system.

Recent advances in genetic manipulation of bacteria paved the way for a new era in the use of probiotics. The incorporation of specific biomarkers to microorganisms leads to bioengineered probiotics, with potential applications for CRC screening and treatment, as probiotics can selectively colonize specific cell types and tumour types[275]. *E. coli* Nissle 1917 (EcN) is a probiotic strain commonly used to treat inflammatory bowel disease[276]. In mice harbouring CRC or colon adenomas, orally delivered bioluminescent EcN specifically colonized the colon tumoural lesions. Importantly, this feature was not related to the production of colibactin by the strain. In a randomized double-blind study, normal EcN administered to patients with CRC before surgery also colonized tumour tissue. Furthermore, 48 h after oral delivery, EcN was undetectable in faecal samples of healthy mice, while it was retained in mice carrying a CRC lesion. Bioengineering an EcN strain able to produce salicylate, a metabolite excreted in the urine, led to the same results, thus allowing the tracking of early colon lesions in stools or urines. Interestingly, oral administration of EcN engineered to produce nanobodies blocking PD-L1 and CTLA-4 targets could reduce the disease burden in mice harbouring colon adenomas through immune-mediated effects. Thus, the ability of EcN to colonize (pre-)tumoural colon lesions could also exert therapeutic effects[277].

In another study, an *E. coli* strain was modified in two ways: (1) to express histone-like protein A with the aim to adhere to CRC cells expressing heparin sulfate, and (2) to transform glucosinolates into sulforaphane, a pro-apoptotic compound. In mice, combining oral ingestion of the strain and glucosinolates led to significant reduction of CRC lesions secondary to chemical colitis[278].

Data accumulate regarding the presence and pro-oncogenic role of specific anaerobic bacteria in the microenvironments of many tumour types[222]. This approach could thus also be leveraged outside gut (pre-) tumoural lesions. Using a different strategy, preclinical proof of interest of engineered probiotics has already been demonstrated to target pancreatic cancer in mice. Here, the authors used a strain of *Listeria monocytogenes* to infect MDSCs, before their attraction to the tumour

BOX 2

Microbiota-based therapies against cancers: preview of current clinical trials

The recent advances demonstrating an association between FMT and modulation of response to anticancer immunotherapy paved the way for many ongoing clinical trials. Most are dedicated to cancers considered targetable by immunotherapy (RCC, NSCLC and melanoma), aiming to reverse or improve poor response to immunotherapy using stool samples from good responders (NCT05286294, NCT05251389, NCT05533983, NCT05669846, NCT06623461, NCT04988841 and NCT06030037). Other trials aim to generate proof of evidence that the potential utility of FMT could embrace other cancer types and be synergistic with other treatment modalities (HCC: NCT06643533, NCT05690048 and NCT05750030; pancreatic cancer: NCT06393400 and NCT04975217; GC: NCT06346093; colorectal adenomas: NCT06205862), or reduce cancer-related cachexia (NCT05606523). FMT could also dampen adverse events related to anticancer therapies, especially (immune-related) colitis (NCT04038619, NCT06206707 and NCT03819296).

Regarding prebiotics, probiotics and postbiotics, most clinical trials are also dedicated to the improvement of anticancer immunotherapeutics (NCT06548789, NCT06466434, NCT05303493, NCT06250335, NCT05821751, NCT06428422 and NCT06398418). Research is also ongoing regarding other cancer types or other treatment options (chemo-radiotherapy in anal cancer: NCT03870607; chemotherapy in colon cancer: NCT04131803; chemotherapy in GC: NCT05901779). Other studies aim to demonstrate their positive impact in the perioperative setting of digestive cancer lesions (NCT05271344 and NCT06456229) or their ability to prevent the recurrence of colon precancerous lesions (NCT05592886). Such complements could also have preventive properties, reducing the risk of immune-related colitis (NCT06508034), chemotherapy-related mucositis (NCT06576986) or endocrine therapy-related vulvo-vaginal atrophy (NCT05562518).

microenvironment. The strain was attenuated and engineered to produce the highly immunogenic tetanus toxin. Its accumulation in the tumour microenvironment led to the recruitment of toxin-specific memory $CD4^+$ T cells, and a $CD4^+$ T cell-mediated tumour lysis. Importantly, this construct also allowed the targeting of metastases[279].

Future research is required to understand if such genetically modified bacteria can engraft in the host microbiota and remain stable and effective over time. Furthermore, this approach raises important questions regarding safety, both for the patient and for the community and the environment.

FMT

FMT involves the transfer of a microbial community to a recipient. FMT is widely known for its efficacy to treat recurrent *Clostridioides difficile* infections and has a well-established safety record for this application[280]. Preliminary findings from a phase I clinical trial (NCT03772899) indicate that FMT from a healthy and cancer-free donor may help overcome primary resistance in patients with advanced-stage melanoma who have not previously been treated with anti-PD-1 antibodies[281]. This FMT was administered before the patients received the anti-PD-1 therapy. Two recent studies demonstrated clinical benefits of FMT in a subset of treated patients including an increased presence of bacterial taxa previously linked to positive anti-PD-1 responses, enhanced activation of $CD8^+$ T cells and a reduced frequency of IL-8-expressing myeloid cells, which have a role in immunosuppression. These findings provide proof-of-concept evidence that FMT can influence the efficacy of immunotherapy in patients with cancer[282,283].

Several other studies are currently investigation with the aim, among others, of potentially overcome ICI resistance in patients with melanoma, RCC and NSCLC receiving first-line ICI treatment[284] (NCT04130763, NCT03341143 NCT04951583, NCT05251389, NCT04758507, NCT04521075 and NCT05008861). However, before moving to a potential universal use of FMT, there are still substantial gaps in the understanding of the mechanisms by which FMT influences immune function and responses. Along with the adequate supportive ecosystem for the microbial community, we will not rule out the potential role of specific viruses and phages in this process[285]. Identifying the most suitable donors will be crucial for the success of future research. Yet, the precise composition of an 'ideal' microbiota that would optimize the response to ICIs, and thus define the ideal donor, is still undetermined (Fig. 2).

Data in other cancer types are less mature, and until now merely descriptive. In mice harbouring chemically induced CRC, FMT has been shown to restore the composition and diversity of the gut microbiota, while also reducing colon inflammation and dysplasia lesions and restoring body weight. In colon cells, FMT reduced pro-inflammatory signalling and increased anti-inflammatory signalling. This was associated with an upregulation of T_{reg} lymphocytes in the tumour microenvironment[286]. In mouse models of pancreatic cancer, transplantation of human faeces originating from long-term survivor patients, but not from short-term survivor patients, induced an antitumour response and activated an anticancer immunity[287]. Further research regarding how FMT induces immune activation in pancreatic cancer is mandatory before considering any clinical trial.

Postbiotics

A postbiotic is a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host"[288] and they represent an emerging area of interest in cancer therapy. Unlike probiotics, which involve the administration of live microorganisms, postbiotics focus on bioactive compounds produced by microorganisms, such as microbial metabolites, cell wall components, bacterial lysates, extracellular vesicles and secreted proteins. These compounds can modulate the immune system, influence metabolic pathways and affect the tumour microenvironment, making them promising candidates for enhancing cancer treatment outcomes, while avoiding some of the concerns linked to live microorganisms.

One of the key advantages of postbiotics is their relatively safer profile compared to probiotics, as they do not carry the risk of bacterial translocation or infection, particularly in immunocompromised patients. Postbiotics can still exert important immunomodulatory effects, promoting anti-inflammatory responses, enhancing immune function, and even stimulating the production of SCFAs and other metabolites that are known to impact tumour growth[289]. Additionally, postbiotics may modulate the activity of antigen-presenting cells such as DCs, leading to enhanced activation of T cells and better immune responses against tumours[290].

Another critical aspect of postbiotics is their role in maintaining gut barrier integrity. By supporting the gut's epithelial cells and reducing inflammation, postbiotics can help prevent the systemic spread of harmful microbial components that may contribute to tumorigenesis[290]. This preservation of gut homeostasis is essential, particularly in patients with cancer undergoing therapies that can disrupt the gut microbiota, such as chemotherapy and radiation. For example, pasteurized *A. muciniphila* (considered as a postbiotic[288]) has been shown to mitigate 5-fluorouracil-induced intestinal mucositis in

BOX 3

Emerging evidence for non-bacterial components of the gut microbiota and cancers

Evidence indicates that non-bacterial components of the gut microbiota, such as fungi, have crucial roles in cancer biology[296]. A landmark study demonstrated that fungal translocation from the gut to the pancreas promotes the progression of PDAC[296]. This study revealed that the fungal microbiota, particularly species of the genus *Malassezia*, colonizes pancreatic tumours and contributes to cancer progression by activating the complement cascade through the mannose-binding lectin pathway. Furthermore, the authors showed that depleting fungi in mouse models of PDAC via antifungal treatment reduced tumour growth, while recolonization with *Malassezia* species restored tumour progression. These findings suggest a potential therapeutic avenue targeting fungal components of the microbiota in PDAC. The study underscores the complex interplay between fungal populations and the immune system, emphasizing that the role of fungi in cancer extends beyond the gut and into the tumour microenvironment. Another study also identified CRC-associated fungal dysbiosis, characterized by an increased Basidiomycota:Ascomycota ratio in patients with CRC compared to healthy control individuals. Fungal class Malasseziomycetes was enriched, while Saccharomycetes and Pneumocystidomycetes were depleted in CRC. Fourteen fungal biomarkers distinguished CRC from controls with high diagnostic accuracy (area under the curve, 0.93), validated in two independent cohorts (area under the curve, 0.82 and 0.74). Ecological analysis revealed more synergistic intrafungal interactions and antagonistic bacterial–fungal correlations in CRC, suggesting that these ecological associations may contribute to colorectal carcinogenesis.

Similarly, the gut virome, particularly bacteriophages, has been associated with CRC. Studies have identified distinct viral signatures in the gut microbiota of individuals with CRC, indicating that changes in the virome may contribute to cancer development[297]. Using 16S rRNA, whole shotgun metagenomics and purified virus sequencing, researchers identified distinct differences in the viromes of patients with CRC, which were dominated by temperate bacteriophages serving as community network hubs. These findings suggest that bacteriophage communities may indirectly influence CRC progression by modulating bacterial host communities, providing foundational evidence for a biological role of the virome in CRC and advancing our understanding of its aetiology.

These findings highlight the complex interplay between various microbial communities and their collective impact on cancer biology. Incorporating discussions on the mycobiome and virome into cancer research provides a more comprehensive understanding of the microbiota's influence on cancer. This holistic approach can reveal novel insights into cancer aetiology and potential therapeutic targets.

tumour-bearing mice[291]. It has also been shown that a purified membrane protein (Amuc_1100) from *A. muciniphila* or the pasteurized form reduces colitis-associated tumorigenesis in mice by modulating $CD8^+$ T cells[267]. Along the same line of interactions between specific postbiotics and microbiota metabolites, one study in mice shows that pasteurized *Akkermansia* was more effective than the live form in raising intestinal concentrations of polyamines, SCFAs, 2-hydroxybutyrate and various BAs, which were also found to be elevated in the bloodstream. These metabolites have been discussed earlier and this Review offers a biochemical explanation for the positive effects of pasteurized *A. muciniphila*.

In pancreatic cancer, analyses of several independent cohorts demonstrate that 3-IAA, a metabolite of tryptophan, is enriched in patients that respond to the main chemotherapy regimens (folfirinox or gemcitabine and nab-paclitaxel). In vivo, providing the metabolite (through FMT or food supplementation) leads to increased tumour control in mouse models of this disease. This is mechanistically linked to the impact of the metabolite on oxidative stress in the tumour microenvironment, a crucial factor controlling the metabolic fitness of the cancer cells[106]. Of high interest, this feature of chemotherapy efficacy modulation through a specific metabolite could be effective outside pancreatic cancer, and not dependent on translocation of bacteria to the tumour site.

Conclusions, outlook and future challenges

The role of the gut microbiota in cancer development and therapy represents an exciting frontier in oncology, as emerging research highlights its profound influence on immune modulation and cancer treatment efficacy, particularly in immunotherapy. The discovery that specific microbial species and metabolites can impact tumour progression, and the effectiveness of ICIs opens new avenues for therapeutic intervention. These findings suggest that targeting the microbiome could enhance patient responses to cancer therapies, offering potential personalized treatment strategies.

One of the most striking aspects of the gut microbiota's impact on cancer therapy is its ability to modulate immune responses. Through interactions with immune cells, the microbiota can influence the outcome of immunotherapy, chemotherapy and other treatments by enhancing or suppressing antitumour activity. Research demonstrates that certain bacterial species, such as *A. muciniphila*, and microbial metabolites, such as SCFAs and tryptophan derivatives, have crucial roles in promoting favourable immune responses. However, the complex interactions between the host immune system, microbiota and tumour microenvironment present considerable challenges in fully understanding and harnessing these relationships.

Several strategies are being explored to manipulate the gut microbiota to enhance cancer treatment efficacy. Dietary interventions, prebiotics, probiotics and FMT are among the most promising approaches. Dietary fibres and prebiotics, for example, have been shown to foster beneficial microbial populations that can improve responses to ICIs. Probiotics and next-generation beneficial bacteria have also demonstrated potential in restoring treatment sensitivity in patients with cancer. Additionally, FMT has been effective in overcoming resistance to immunotherapy in certain patients with advanced cancers. However, these therapeutic strategies remain in their infancy. A key challenge lies in identifying the optimal microbial compositions that can enhance treatment outcomes. The variability of gut microbiota across individuals, influenced by factors such as genetics, diet, lifestyle and sampling methods (Box 1) complicates efforts to standardize these therapies. Furthermore, the long-term effects of manipulating the microbiome on cancer progression and recurrence are not yet fully understood, and large-scale clinical trials are required to validate initial findings and explore safety concerns.

Despite substantial advances, several challenges remain in translating microbiota research into clinical practice. First, the high degree of interindividual variability in microbiota composition means that responses to dietary interventions or microbial therapies can vary widely between patients. This variability complicates the development of standardized microbiota-based therapies, making it difficult to predict patient responses and optimize treatment strategies. Second, although certain bacterial species and metabolites have been linked to

improved cancer therapy outcomes, the precise mechanisms by which microbiota influence immune responses and tumour biology are not yet fully elucidated. Many findings discussed in this Review remain at the level of correlative descriptions, missing further evidence of a causal relationship. A more detailed understanding of these mechanisms is needed to refine therapeutic approaches and develop more effective, targeted interventions. Third, FMT, although showing promise in clinical trials (Box 2), faces challenges related to donor selection and the identification of ideal microbial compositions. The safety and consistency of FMT as a cancer treatment also require further investigation, particularly with regard to long-term effects and potential adverse outcomes. Finally, although manipulating the microbiota has shown potential in improving cancer treatment efficacy, there is still a need for more research to address the ethical and regulatory considerations surrounding microbiota-based therapies. Developing guidelines for the use of such interventions in clinical settings is critical to ensuring patient safety and maximizing therapeutic benefit[292].

In conclusion, the gut microbiota represents a promising yet complex frontier in cancer treatment, but emerging evidence for non-bacterial components of the gut microbiota and cancers should also be considered (Box 3). Although early findings highlight its potential to enhance the efficacy of cancer therapies, numerous challenges remain in fully understanding and manipulating the microbiome to optimize treatment outcomes. Moving forward, large-scale clinical trials and further research into the mechanisms of microbiota–immune interactions will be crucial to unlocking the full therapeutic potential of the gut microbiota in oncology. The development of personalized microbiota-based therapies could revolutionize cancer treatment, but this will require overcoming important scientific, clinical and regulatory challenges.

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Author contributions

A.N., C.v.M., B.F.J., M.V.H. and P.D.C. conceived and designed the Review. P.D.C. coordinated and supervised the Review. A.N., C.v.M., B.F.J., M.V.H. and P.D.C. performed the literature review. A.N., C.v.M., B.F.J., M.V.H. and P.D.C. conducted the writing. M.V.H. prepared the figures. A.N., C.v.M., B.F.J., M.V.H. and P.D.C reviewed the final draft. All authors discussed the Review, reviewed the revised versions, commented on the manuscript and figures before submission, and agreed with the final submitted manuscript.

Competing interests

P.D.C. and B.F.J. are inventors on patent applications dealing with the use of gut bacteria and their components in the treatment of diseases. P.D.C. was a co-founder of The Akkermansia Company and Enterosys. The remaining authors declare no competing interests.

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