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Review article

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Towards modulating the gut microbiota to enhance the efficacy of immune-checkpoint inhibitors

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Abstract

The gut microbiota modulates immune processes both locally and systemically. This includes whether and how the immune system reacts to emerging tumours, whether antitumour immune responses are reactivated during treatment with immune-checkpoint inhibitors (ICIs), and whether unintended destructive immune pathologies accompany such treatment. Advances over the past decade have established that the gut microbiota is a promising target and that modulation of the microbiota might overcome resistance to ICIs and/or improve the safety of treatment. However, the specific mechanisms through which the microbiota modulates antitumour immunity remain unclear. Understanding the biology underpinning microbial associations with clinical outcomes in patients receiving ICIs, as well as the landscape of a 'healthy' microbiota would provide a critical foundation to facilitate opportunities to effectively manipulate the microbiota and thus improve patient outcomes. In this Review, we explore the role of diet and the gut microbiota in shaping immune responses during treatment with ICIs and highlight the key challenges in attempting to leverage the gut microbiome as a practical tool for the clinical management of patients with cancer.

Sections

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Key points

- Growing evidence supports a role for the gut microbiota in shaping both antitumour immune responses and the development of toxicities during treatment with immune-checkpoint inhibitors (ICIs).
- Targeting the gut microbiota provides a potentially powerful tool to overcome resistance to ICIs and/or to reduce the risk of severe toxicities.
- Interindividual microbial heterogeneity poses a major challenge to the study of the microbiota across human populations and to the translation of microbial findings into the clinic.
- Interventions designed to modulate the microbiota could have profound effects in augmenting the effectiveness of ICIs in a subset of patients, although this strategy is unlikely to be uniformly effective; identifying which patients will benefit is an important step.
- A personalized approach guided by a patient's baseline gut microbiota, systemic immune parameters and tumour characteristics will be important in order to optimally target the microbiota and thus improve the outcomes of patients receiving ICIs.

Introduction

The role of the immune system in immunosurveillance and the control of tumour growth is now well established. Tumour growth and progression are often associated with a dysfunctional or exhausted antitumour immune response, in which upregulation of inhibitory molecules such as immune checkpoints or immunosuppressive cytokines/chemokines restrict the antitumour activity of leukocytes[1]. A range of immune inhibitory molecules are therefore key therapeutic targets. Immune-checkpoint inhibitors (ICIs) targeting PD-1, PD-L1 or CTLA4 are established as effective treatments for many patients with cancer, particularly those with advanced-stage melanoma, renal cell carcinoma (RCC) and non-small-cell lung cancer (NSCLC)[2–6]. Even with the most effective immunotherapeutic strategy available to date, the combination of ICIs targeting PD-1 and CTLA4, just over 40% of patients with advanced-stage melanoma still die from their disease owing to resistance[7]. Moreover, treatment-induced immune-related adverse events (irAEs) frequently cause morbidities, which are often severe[8–10]. These autoimmune or autoinflammatory manifestations often limit both the use and effectiveness of this therapeutic approach.

The gut microbiota, which consists primarily of bacteria along with other microbes including Archaea, viruses and fungi that populate the lumen and mucosa of the gastrointestinal tract, has attracted much interest over the past decade owing to the potential to modulate antitumour immunity. The intestinal microbiota has a key role in the maintenance of gut homeostasis and general health by participating in a diverse range of physiological functions with both local and systemic effects[11,12]. Locally, the microbiota maintains intestinal barrier function and regulates mucosal immunity, which in turn protects the host from infection with pathogens and excessive exposure to commensals. More systemically, the gut microbiota affects the regulation of metabolism, inflammation, haematopoiesis and immunity[13,14], and has a pivotal role in both the development and function of the immune system[15]. Dysregulation of the gut microbiota has been implicated in the development of a variety of immune-driven diseases throughout the body, including inflammatory bowel disease, asthma, allergies, diabetes and some cancers[16–18]. It is therefore likely that the gut microbiota also influences the susceptibility to developing severe autoimmune or autoinflammatory irAEs following treatment with ICIs.

The demonstrated systemic effects of the gut microbiota in shaping immune responses[11,12,15] indicate that an individual's 'baseline' microbial community is not only fundamental to immune homeostasis, but is also likely to influence tumour immunosurveillance, how an individual will respond to ICIs, and whether unintended destructive immune pathologies will accompany treatment with such agents. Diet is a key environmental factor that shapes the composition and function of the gut microbiota. Diet is closely linked to the capacity of the microbiota to maintain intestinal barrier integrity and regulate immune homeostasis. In this Review, we discuss diet–microbiota interactions and their implications for immune function, antitumour immunity and responses to ICIs, along with strategies for modulating the gut microbiota that might improve the activity of these agents.

Antitumour immunity and ICIs

The immune system has a protective role against the development of tumours; however, tumour cells can acquire the ability to evade detection by the immune system, leading to failures in immunosurveillance and ultimately tumour growth and progression[19,20]. ICIs that target PD-1, PD-L1 or CTLA4 aim to ameliorate tumour-induced immunosuppression by removing the suppression of cellular antitumour immunity mediated by these checkpoints and their ligands. In patients with a response to ICIs, reversing these effects enables reactivation of the antitumour immune response followed by clearance of tumour cells by the immune system[21].

Several tumour-intrinsic parameters, including baseline tumour cell PD-L1 expression, neoantigen load, a pre-existing IFNγ signature and the presence of tumour-infiltrating lymphocytes, are predictive of a response to ICIs[22–27]. These features are a function of a 'T cell inflamed' microenvironment and indicate pre-existing antitumour immunity, despite ongoing suppression of tumour-specific T cell function[26–28]. Development of this antitumour immune 'awareness' is also dependent on several tumour-extrinsic factors, such as the functional capacity of the immune system itself, which is governed by host and environmental factors including germline genetics, the gut microbiota and diet[29]. Thus, the immune system of some individuals is better primed to respond to their tumour upon reactivation of cell-mediated antitumour immunity following treatment with ICIs.

Treatment with one or more ICIs poses a fundamental challenge to immune regulation. This challenge explains the propensity for ICI-associated irAEs that occur owing to the reactivation of self-reactive immune cells in non-malignant host tissues[30–32]. These inflammatory processes are not only debilitating for patients but can also influence the effectiveness of ICIs, sometimes resulting in patients having to discontinue or even cease treatment and/or requiring immunosuppression. Therefore, not all inflammation is necessarily beneficial for antitumour immunity and the type that is induced is likely to be highly relevant. For example, aberrant exposure to microbial products might drive polarization towards T helper 2 cell (T_H2) and T_H17 responses, rather than towards T_H1 responses that enable optimal tumour clearance, and might therefore be counterproductive[15,21]. Fundamentally, the ideal microbial state for an effective immune response during treatment with ICIs is one that facilitates a balance between immune stimulation that promotes tumour clearance and immune

regulation that provides an adequate level of protection from excessive immune activation in the form of irAEs.

Role of diet–microbiota interactions in immunity

Diet and the gut microbiota are important factors that shape immune fitness. Strong evidence of the role of the gut microbiota in shaping immune function is provided by studies involving germ-free and antibiotic-treated mouse models[33]. Germ-free mice have impaired development of intestinal lymphoid tissues, including Peyer's patches and isolated lymphoid follicles, with consequent deficiencies in secretory IgA levels, which are essential for the regulation of intestinal homeostasis[34,35]. Furthermore, the immune systems of germ-free mice are often polarized towards pro-allergic–T_H2-type immune phenotypes with higher systemic IgE levels and increased susceptibility to orally induced anaphylaxis[36,37], thus highlighting the importance of the microbiota in establishing immune regulation during early development. Perturbations of the microbiota during adulthood can lead to dysregulated immune responses and the development of autoimmune or auto-inflammatory diseases, thus highlighting the systemic effects of microbial dysbiosis[38]. In mouse models of arthritis and autoimmune encephalitis, T_H17 and/or T follicular helper (T_{FH}) cells differentiate in response to colonization with particular host-specific gut microbes (such as segmented filamentous bacteria) and migrate to the spleen where they support the formation of germinal centres followed by the production of autoantibodies, thus driving the emergence of an autoimmune pathology[39–41].

The balance of effector versus regulatory T cells (T_{reg}) in the gut is critical for maintaining immune homeostasis and limiting the extent of inflammation. The gut microbiota is central to regulating this balance and shaping the intestinal T cell repertoire[42]. The gut microbiota is also a dominant source of cognate antigens for T cell activation. Such interactions drive the generation of adaptive immune responses towards specific gut bacterial antigens[43–45]. Not all microbial species can induce adaptive immune responses, although perturbations to the gut microbiota (for example, via the administration of antibiotics) result in major shifts in the T cell repertoire[42]. Diet has also been shown to influence interactions between T cells and their cognate antigens by altering antigen expression[45]. Similarly, the IgA repertoire is shaped to bind the 'self' microbiota and is able to respond rapidly to alterations in this ecosystem[46] including the recognition of single species and/or individual strains[47].

Diet is a key factor that shapes the composition and function of the microbiota[48], which can also directly modulate immune function[49,50]. The production of metabolites from dietary nutrients is a prominent means by which microbes modulate systemic immunity. The overall output of bacterial metabolites in the gut is dependent on the type of microbes present as well as the nutrients ingested and utilized by these microbes, as described in detail elsewhere[51,52]. These metabolites can influence host immune cell activity by direct signalling (for example, through activations of metabolite-sensing G protein coupled receptors (GPCRs)) or indirectly by altering immune cell contact with microbes through regulation of gut permeability[53,54].

Short-chain fatty acids (SCFAs) such as butyrate, acetate and propionate have been the focus of numerous studies demonstrating the ability of microbial metabolites to modulate the immune system. SCFAs are produced by the fermentation of indigestible dietary fibre by specific bacteria located in the colon[55]. Butyrate and propionate are predominantly utilized locally in the gut or liver, whereas acetate is readily detected in the systemic circulation, suggesting that these metabolites might affect immune function at more distant sites[38,54,56]. SCFAs are also important for maintaining gut barrier integrity. SCFAs can bind GPR43 on intestinal epithelial cells resulting in NLRP3 inflammasome activation and IL-18 production, which promotes epithelial barrier repair and turnover[57]. Butyrate is also a primary source of energy for colonocytes and is therefore important for supporting epithelial cell renewal[56,58]. Maintaining barrier integrity is not only critical for maintaining intestinal homeostasis but can also limit systemic inflammation by preventing aberrant exposure to microbial products[38]. Dietary fibre intake has been found to be protective against the development of both colitis and colorectal cancer in a butyrate-dependent manner both in mouse models and in a dietary intervention study[57,59,60]. The tumour suppressive capacity of butyrate, which inhibits the proliferation and survival of cancer cells via GPR43 signalling and/or inhibition of histone deacetylase (HDAC), has been demonstrated across multiple cancer cell lines[55,58,61–63]. SCFAs have also been shown to promote T_{reg} differentiation both directly by inhibiting HDACs[64–66] and indirectly through modulation of $CD103^+$ dendritic cells (DCs) in a GPR43–GPR109A-dependent manner, which are responsible for the induction of T_{reg} differentiation in the gut[67]. Furthermore, signalling via metabolite-sensing GPCRs can inhibit NF-κB and thus prevent the production of pro-inflammatory cytokines by both immune and epithelial cells[54,68].

The consumption of suboptimal diets, such as a Western-style diet characterized by high levels of saturated fats and low levels of fibre, has been linked to dysregulation of the gut microbiota and a reduction in SCFA levels. Such diets have also been implicated in a wide variety of inflammatory and metabolic diseases[56,58,69,70]. For example, a high-fat diet has been demonstrated to suppress MHC class II expression on intestinal epithelial cells and promote intestinal tumour development in a mouse model, highlighting the effects of diet on immunosurveillance[71]. In another study, a low-protein diet was shown to promote tumour immunosurveillance. In this study, a reduction in amino acid availability induced IRE1α-dependent endoplasmic reticulum stress in tumour cells in three independent mouse models, leading to increased IFNγ production and $CD8^+$ T cell infiltration[72], suggesting that diet can also directly alter the metabolic capacity of a tumour.

Fibre is an important source of nutrients for the gut microbiota in the large intestine, although some microbes are capable of utilizing host-derived glycans or mucins as alternative nutrient sources. For example, certain gut microbes, such as *Akkermansia muciniphila* and *Barnesiella intestinihominis*, are able to exclusively utilize mucins (and are referred to as 'mucin specialists'), whereas other microbes exhibit metabolic flexibility and can use a variety of energy sources (and are referred to as 'mucin generalists'). *Bacteroides thetaiotaomicron*, for example, can switch to utilizing host-derived glycans when dietary carbohydrates are unavailable[73,74]. Mucin turnover is critical for the maintenance of intestinal integrity. This process involves a tight balance between mucus degradation and renewal. *Muc2*-deficient mice have enhanced levels of colonic inflammation and exacerbated dextran sulfate sodium (DSS)-induced colitis compared with wild-type mice, and it is likely that this reflects the closer proximity of bacteria to the intestinal epithelium owing to a thinner mucus layer[75,76]. A symbiotic relationship with mucin-degrading microbes occurs in the context of a high-fibre diet, thus supporting mucin turnover and promoting barrier function. However, in the context of fibre deprivation, the mucin-degrading microbiota can erode the mucus layer leading to impaired barrier function, thus promoting intestinal inflammation

and susceptibility to pathogens[74]. The dietary context and the overall content of the microbial ecosystem are therefore highly relevant to the function of microbes, and subsequently their immunomodulatory effects (Fig. 1).

The gut microbiota in patients receiving ICIs

Antitumour immunity and responsiveness

Evidence now exists for a role of the gut microbiota in determining responsiveness to ICIs. Data from several seminal preclinical studies

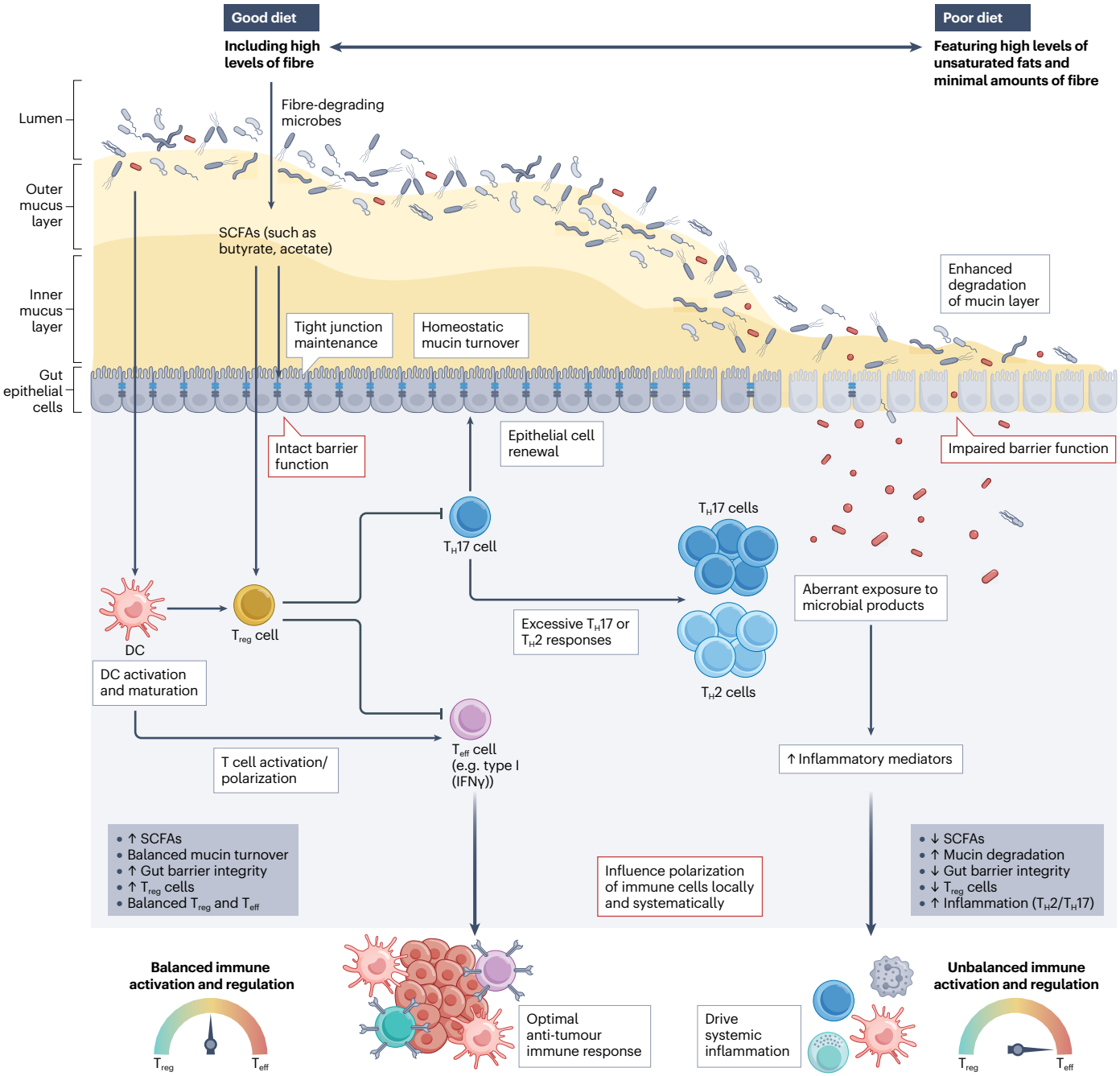

Fig. 1 | Interactions between diet, the gut microbiota and antitumour immunity. Diet influences the balance between fibre-degrading and mucin-utilizing bacteria in the gut microbiota. In turn, this balance influences mucin turnover and the production of short-chain fatty acids (SCFAs), which promote epithelial cell renewal and gut barrier integrity. Optimal barrier function supports a balance between immune activation and regulation, which promotes immune homeostasis. This process prevents excessive T_H17 immune responses and aberrant exposure to microbial products, which can drive pathogenic inflammation. By contrast, a prolonged period of consumption of a low-fibre diet can result in enhanced mucin degradation and impaired barrier function, that increases exposure to microbial products and promotes excessive inflammation in the gut. The balance of effector (T_{eff}) versus regulatory T cells (T_{reg}) in the gut is critical for limiting inflammation locally, which can alter systemic immune polarization and function. DC, dendritic cell; T_H2, T helper 2 cell; T_H17, T helper 17 cell.

demonstrate that the intestinal microbiota can modulate antitumour immunity and is necessary to facilitate the antitumour activity of these agents, with germ-free or antibiotic-treated mice having impaired tumour control[77–79]. Genetically similar mice harbouring distinct commensal gut microbes also have altered tumour growth, suggesting that differences in spontaneous antitumour immunity reflect differences in the microbiota[78]. Notably, the superior responses to ICIs seen in certain mice can be transferred to others via faecal microbiota transplantation (FMT), co-housing or recolonization with more-favourable microbes[78,79].

Data from landmark clinical studies have further established a role of the gut microbiota in responsiveness to ICIs[80–82]. Notably, FMT from patients who were either responsive or not responsive into mice also transferred the patients' response characteristics[80,82], thus establishing a causal link between the microbiota and antitumour immune responses during treatment with ICIs[80–82]. No clear consensus exists on the identity of specific taxa associated with a response to ICIs, although several common themes emerging across clinical studies include the importance of microbial α-diversity, *A. muciniphila*, and species within the *Bifidobacterium* and *Bacteroides* genera, and the Ruminococcaceae family (such as *Faecalibacterium prausnitzii*)[80–89] (Table 1). These discrepancies might arise from several potentially confounding findings including clinical heterogeneity (disease stage, type of ICI received and/or line of treatment), definition of response, cancer type or subtype, geographical variations and small cohort sizes, as well as variations in certain technical factors such as sample collection and storage. As a result, efforts are now focused on unifying microbial signatures to better understand the complex relationship between the microbiota and the clinical outcomes in patients receiving ICIs[85,87–89]. These studies involve large cross-cohort analyses and/or geographically dispersed clinically homogeneous cohorts (varying in size from 218 to 438 patients), and demonstrate that neither specific individual taxa nor microbial α-diversity are universally predictive of response. Notably, some previously reported associations were reproduced in these larger cohorts, although cohort-specific variability was noted and might be linked with certain geographical variations in diet. For example, taxa within the *Bifidobacterium* genus were enriched in responders in two meta-analyses[87,88] and *A. muciniphila* was also associated with response in two of these four analyses (one of which also found positive associations between *Bifidobacterium* species and response)[87,89]. Links between fibre-degrading or butyrate-producing taxa and response were identified across all studies, and involved species within the Ruminococcaceae or Lachnospiraceae families (commonly *F. prausnitzii* and *Roseburia*)[85,87–89]. In line with this observation, higher dietary fibre intake was associated with improved progression-free survival (PFS)[85] and response rates[89] in two of the analyses and these associations also applied to higher microbial α-diversity[85,89]. Links between unfavourable microbiomes, enhanced systemic inflammation and poor response rates were also observed in two analyses[88,89].

Overall, the conclusions of these studies highlight the profound heterogeneity of the gut microbiota and its complex relationship with outcomes in patients receiving ICIs, which clinical tools will need to capture in order to be truly effective. These emerging data suggest the potential clinical utility of signatures combining panels of different microbial species and/or that account for microbial community assemblages or baseline diet; however, further work in this area is required to optimally leverage the microbiome in the predictive setting. The ideal microbial composition and the underlying mechanisms through which the microbiota shapes antitumour immunity are currently only partially understood. Insights obtained to date suggest the existence of a variety of mechanisms, including the production of metabolites or microbial products that act as a source of antigenicity and/or that can have an adjuvant role in modulating systemic immune function. Nonetheless, fundamental questions remain relating to the relative importance of the baseline microbiota in shaping or priming pre-existing anticancer immunity versus how the gut microbiota or subsequent microbial alterations occurring during treatment with ICIs can influence immune reactivation.

Cross-reactivity of microbial and tumour antigens

Molecular mimicry of self and microbial peptides can lead to antigen-specific activation of autoreactive immune cells, which can traffic to distant sites and drive autoimmunity. This effect has been reported in the context of autoimmune uveitis, cardiomyopathy and multiple sclerosis[90–92]. Through a similar principle, microbiota-specific T cells might recognize tumour-associated antigens. For example, cross-reactivity has been demonstrated between $CD8^+$ T cells that are specific for enterococcal bacteriophages and MHC class I-restricted tumour antigens[93]. Furthermore, T cells specific for an epitope expressed by *Bifidobacterium breve* have been demonstrated to cross-react with a neoantigen expressed by the B16.SIY melanoma cell line[94]. Tumours in mice lacking *B. breve* consequently grew faster in this study. Memory T_H1 recall responses directed against specific bacteria, including *A. muciniphila*, *Bacteroides fragilis*, *B. thetaiotaomicron* and *Enterococcus hirae*, have been associated with improved responses to ICIs and prolonged PFS in patients receiving ICIs[79,81,95]. Adoptive transfer of *B. fragilis*-specific memory T cells into germ-free or antibiotic-treated mice enabled partial restoration of tumour control by ICIs targeting CTLA4 (ref. 79). These memory responses against commensals might reflect bacterial translocation associated with disrupted barrier function. Alternatively, sampling of mucosal antigens by intestinal DCs could result in the generation of commensal-specific memory responses, without compromising gut integrity[53,96]. In line with this observation, many of the immunomodulatory bacteria associated with enhanced immune activation actively colonize the mucosa as opposed to the intestinal lumen[79,95,97].

Effects on immune activation and function

The microbiota can also have an auxiliary role in promoting antitumour immunity. Microbial-derived products such as microbe-associated or pathogen-associated molecular patterns (including lipopolysaccharide (LPS), peptidoglycans and nucleic acids), can act as signals that promote immune cell activation and function[98,99].

Early evidence of the ability of microbes to influence the degree and polarity of immune activation was provided by studies of the effects of chemotherapy. For example, translocated *E. hirae* are able to induce the polarization of immune cells in secondary lymphoid organs towards a T_H1/IFNγ phenotype, leading to increased ratios of intratumoural cytotoxic T cells to T_{reg} in mouse models[95,100]. The microbiota is also able to prime myeloid cells to produce pro-inflammatory cytokines and reactive oxygen species (ROS) in mice exposed to CpG oligodeoxynucleotides (which are designed to mimic the activation of innate immunity mediated by pathogenic viruses and/or bacteria) and platinum-based chemotherapy. Germ-free and antibiotic-treated mice had impaired responses to these agents, although oral gavage with LPS abrogated the effects of antibiotics, suggesting that the intestinal microbiota modulates inflammatory processes in the tumour microenvironment (TME) via TLR4-dependent signalling[77]. Elsewhere,

Table 1 | Emerging features of the microbiota and their roles in response to ICIs

Ecosystem feature	Clinical evidence	Preclinical evidence
Akkermansia muciniphila	Higher relative abundance in responders to anti-PD-1 (± anti-CTLA4) antibodies in patients with melanoma, NSCLC or RCC[81,87,89]	Oral administration of *A. muciniphila* restores the antitumour activity of anti-PD-1 antibodies in germ-free or antibiotic-treated mice[81]
	Circulating memory T cell responses to *A. muciniphila* associated with improved PFS[81]	Can produce cyclic di-AMP and induce STING type I IFN innate cell reprogramming in the TME to enhance the efficacy of anti-PD-1/PD-L1 antibodies[105]
Bacteroides (*B. caccae*, *B. fragilis*, *B. thetaiotaomicron*)	*B. caccae* and *B. thetaiotaomicron* enriched in patients with melanoma with a response to anti-PD-1 (± anti-CTLA4) antibodies[79,84]	Oral gavage with *B. fragilis* or adoptive transfer of *B. fragilis*-specific memory T cells partially restores tumour control in the presence of anti-CTLA4 antibodies in germ-free or antibiotic-treated mice[79]
		B. fragilis outgrowth following treatment with anti-CTLA4 antibodies mediates a response in mouse models of melanoma that received FMT from human patients[79]
Bifidobacterium (*B. longum*, *B. pseudolongum*, *B. breve*)	*B. longum* more abundant in patients with melanoma with a response to anti-PD-1 antibodies[80]	Oral administration of *Bifidobacterium* spp. alone or in combination with an anti-PD-L1 antibody improves tumour control[78]
		Bifidobacterium spp.-treated mice have enhanced expression of genes involved in antigen presentation, T cell activation and type I IFN signalling[78]
		Reconstitution of germ-free mice with stool samples from patients with a response (high levels of *B. longum*) improves the activity of anti-PD-L1 antibodies[80]
		B. breve-specific T cells cross-react with neoantigens expressed by the B16.SIY melanoma cell line, constraining tumour growth[94]
		B. pseudolongum enhances responsiveness to ICIs through production of inosine[104]
Enterococcus (*E. hirae*, *E. faecium*)	*E. hirae* and *E. faecium* enriched in patients with melanoma or NSCLC with a response to anti-PD-1 antibodies[80,81]	*Enterococcus* species improve the activity of anti-PD-L1 antibodies in mouse models of melanoma via immunologically-active peptidoglycan muropeptides[102]
	Circulating memory T cell responses to *E. hirae* associated with improved PFS[81]	Translocated *E. hirae* induce the polarization of immune cells in secondary lymphoid organs towards T_H1/IFNγ-type phenotypes in mice exposed to cyclophosphamide-based chemotherapy[95,100]
Faecalibacterium prausnitzii	Higher relative abundance in patients with melanoma with a response to anti-PD-1 (± anti-CTLA4) antibodies[82–84,89,111]	Mice receiving FMT from ICI responders have improved responses to anti-PD-L1 antibodies, linked with a greater abundance of *Faecalibacterium*[82]
	Enriched in patients with higher levels of dietary fibre intake[85]	
	Higher levels of intratumoural $CD8^+$ T cells in patients with a microbiota enriched with *F. prausnitzii*[82]	
	Higher levels of *F. prausnitzii* associated with longer PFS durations[82]	
Ruminococcaceae	Ruminococcaceae-dominated microbiomes are associated with higher response rates among patients with melanoma receiving anti-PD-1 (± anti-CTLA4) antibodies[82,88,89]	High-fibre diet improves the activity of anti-PD-1 antibodies[85]
	Associated with higher dietary fibre consumption and improved PFS[85]	

FMT, faecal microbiota transplantation; ICI, immune-checkpoint inhibitor; NSCLC, non-small-cell lung cancer; PFS, progression-free survival; RCC, renal cell carcinoma; T_H1, T helper 1 cell; TME, tumour microenvironment.

a mixture of 11 immunostimulatory bacterial strains that are known to actively colonize the gut mucosa was able to systemically induce IFNγ-producing $CD8^+$ T cells in a mouse model. This immunomodulatory effect was observed both locally and systemically, with higher frequencies of $IFN\gamma^+CD8^+$ T cells observed in organs located beyond the intestines including within tumours[101]. These immune cells were phenotypically distinct from the colonic immune cell populations and their induction was independent of innate signalling, leading the authors to hypothesize that bacterial metabolites rather than bacterial translocation or immune cell migration were responsible for this effect. Data published in 2021 indicate that *Enterococcus* species that promote responsiveness to ICIs have distinctive peptidoglycan remodelling capabilities[102]. These species express a peptidoglycan hydrolase (SagA) that generates cell wall-derived muropeptides capable of stimulating the immune system in a NOD2-dependent manner that enhances the accumulation of cytotoxic $CD8^+$ T cells and pro-inflammatory

monocytes in the TME. Furthermore, NOD2-active muropeptides were found to improve the antitumour activity of ICIs, thus highlighting the potential of synthetic molecules and/or microbial-derived products as adjuvants to enhance the effectiveness of these agents[102,103].

Microbes can also support T cell priming and effector function by modulating the activation and maturation of DCs. *Bifidobacterium* species, *B. fragilis* and *A. muciniphila* have all been found to stimulate DCs to produce IL-12, a cytokine that promotes T_H1 responses[78,79,81]. Similarly, reconstitution of antibiotic-treated mice with *A. muciniphila* in combination with an anti-PD-1 antibody promotes the accumulation of $CD4^+$ central memory T cells expressing the small intestine homing chemokine receptors CCR9 and CXCR3 in both the mesenteric lymph nodes and tumour cells. These central memory cells were found to form intratumoural granulomas, thus increasing the ratio of $CD4^+$ to $FoxP3^+$ T cells in the TME and restoring the antitumour activity of the anti-PD-1 antibody[81]. *Bifidobacterium* spp. have also been found to alter the functional capacity of DCs, with DCs derived from mice exposed to these organisms having greater expression of genes involved in antigen presentation, T cell activation and type I IFN signalling. Functionally, these DCs are able to induce $CD8^+$ T cell proliferation and IFNγ production, which might explain the increased accumulation of $CD8^+$ T cells in the TME in these models[78]. These data are supported by the observation of higher levels of $CD8^+$ T cells and markers of antigen presentation in the TME of patients with a microbiota enriched with *Faecalibacterium* species[82]. Together these data provide strong support for the idea that bacteria-derived signals can modulate DC activation and improve the effector functions of tumour-specific $CD8^+$ T cells.

Metabolites that promote type 1 antitumour immunity

Microbes are known to modulate antitumour immunity via the production of metabolites. Data published in 2020 indicate that inosine, a bacterial purine metabolite, can promote T_H1 activation via adenosine receptor ($A_{2A}R$) signalling, which improves the activity of ICIs across mouse models of several different cancer types[104]. *Bifidobacterium pseudolongum* and *A. muciniphila* are both able to produce this metabolite. Hypoxanthine and xanthine, and other related metabolites, are also elevated in the serum of mice colonized with *B. pseudolongum*[104]. Interestingly, hypoxanthine and inosine monophosphate levels are also elevated in mouse serum following inoculation with the previously mentioned 11-strain microbial consortium reported to induce IFNγ-producing $CD8^+$ T cells[101]. Microbial production of STING agonists has also been shown to induce monocytes in the TME resulting in type I IFN production and skewing the polarization of innate immune cells towards an anti-tumorigenic phenotype[105]. A high-fibre diet or FMT from patients with a response to ICIs are both able to induce a similar STING type I IFN innate cell reprogramming effect in the TME and enhance the efficacy of ICIs targeting PD-1 or PD-L1 immunotherapy. Notably, *A. muciniphila* has been shown to produce cyclic di-AMP (a naturally occurring STING agonist) that can promote the occurrence of this phenotype[105]. Trimethylamine *N*-oxide (TMAO) is another microbial metabolite that has been demonstrated to promote $CD8^+$ T cell-mediated antitumour immunity via induction of pyroptosis, leading to enhanced activity of anti-PD-1 antibodies in mouse models of triple-negative breast cancer[106]. In another study, TMAO was found to enhance antitumour immunity in mouse models of pancreatic ductal adenocarcinoma (PDAC), albeit by inducing an immunostimulatory phenotype in macrophages that potentiated type I IFN signalling and T cell effector function[107]. A choline-rich diet or dietary supplementation with choline (a TMAO precursor) also enhanced tumour control with an ICI[106,107]. The microbiota-derived tryptophan metabolite indole-3-acetic acid (3-IAA) has also been shown to enhance the activity of chemotherapy in mouse models of PDAC by promoting the accumulation of ROS in cancer cells, ultimately to cytotoxic levels[108]. Interestingly, the 3-IAA producers *B. fragilis* and *B. thetaiotaomicron* were both enriched in patients with PDAC who had a response to chemotherapy, and the presence of these organisms has also been linked with antitumour immunity in patients receiving ICIs[79,84,108].

F. prausnitzii and other members of the Ruminococcaceae family have been associated with response in patients receiving ICIs across several studies[82–84,89]. These microbes are key fermenters of dietary fibre resulting in the production of butyrate, which is essential for maintaining both epithelial integrity and intestinal homeostasis[56,58]. Data from several studies indicate that the levels of these beneficial microbes are reduced in the context of inflammatory bowel disease and colorectal cancer[69]. However, evidence on the implications of SCFAs for response to ICIs is conflicting, suggesting a dependence on a specific physiological context. For example, high concentrations of faecal SCFAs have been associated with both responsiveness and longer PFS in patients with various solid tumours[109,110], although higher concentrations of serum SCFAs have been associated with shorter PFS[111]. Oral administration of butyrate increases systemic SCFA levels and limits the antitumour activity of anti-CTLA4 antibodies in mouse models by restraining the induction of tumour-specific T cells in the tumour-draining lymph nodes, with evidence of a similar effect in patients with higher serum SCFA levels[111]. Conversely, an orally administered gel that enables prolonged release of the dietary fibre inulin is able to modulate the microbiota, leading to increased faecal SCFA levels and enhanced systemic antitumour immunity in mouse models exposed to anti-PD-1 antibodies[112]. This finding can be rationalized by the knowledge that systemic SCFA levels are influenced by both microbial production in the gut and consumption by colonocytes (particularly butyrate). Furthermore, the systemic accumulation of orally administered SCFAs is also dependent on absorption in the small intestine[113]. In line with this finding, oral administration of free SCFAs did not improve the efficacy of ICIs in this study, in contrast to the administration of inulin gel that is metabolized by the gut microbiota[112]. However, in terms of potentially beneficial impacts, it was proposed that SCFAs enhance the memory potential of antigen-primed $CD8^+$ T cells and promote their differentiation into stem-like $Tcf1^+PD\text{-}1^+CD8^+$ T cells both in the mesenteric lymph nodes and in the TME during PD-1 blockade, promoting tumour control[112,114]. This observation is consistent with the findings of a previous study showing that butyrate can enhance the memory potential of activated $CD8^+$ T cells, which is particularly relevant in the context of immunotherapies that rely on the reactivation of pre-existing, albeit functionally suppressed, antitumour immune responses[115].

It is likely that the microbiota is able to promote responsiveness to ICIs through several mechanisms that are probably not mutually exclusive (Fig. 2). Although further research is required, the available data suggest that specific microbes can skew the polarization of immune cell populations towards type 1 immunity, which promotes tumour clearance. Thus, manipulating the microbiota might enable the reprogramming of defective type 1 antitumour immune responses to improve the efficacy of treatment in some patients.

irAEs

Beyond modulating antitumour immunity, it is likely that the gut microbiota also influences susceptibility to the more severe autoimmune and/or inflammatory adverse effects of ICIs. These toxicities can

become manifest in any organ, most commonly in the colon, liver, skin, thyroid and lungs. Notably, many of these toxicities occur at key epithelial barrier sites[116]. The central role of tissue-resident immune cells in many of these locations suggests that the pre-established state of the local immune environment has important implications for the development of irAEs[97,117]. Data from several studies demonstrate associations

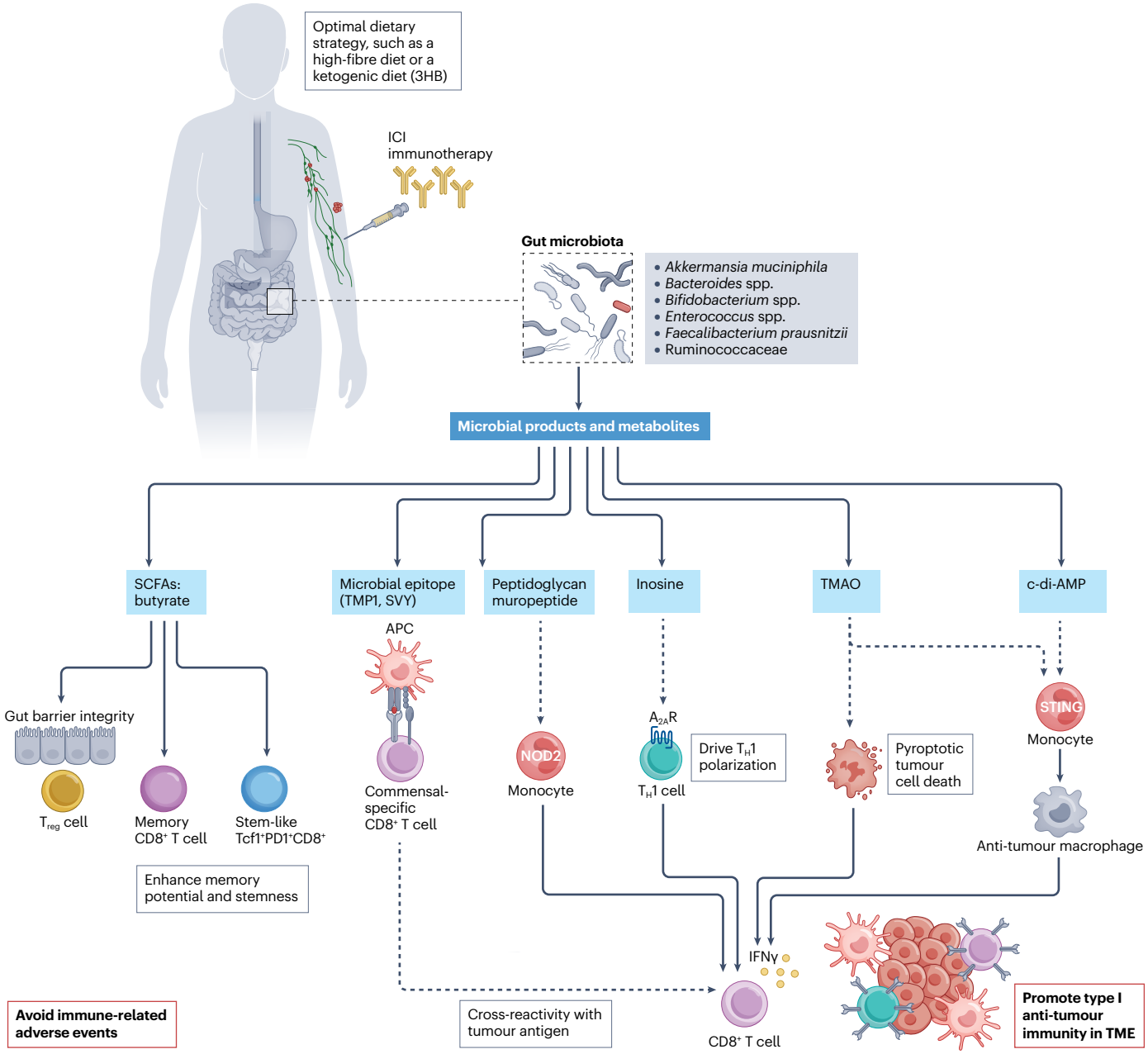

Fig. 2 | Putative mechanisms of interactions between microbes, immune cell subsets and immune-checkpoint inhibitors. Several candidate species have been demonstrated to promote antitumour immunity and enhance the activity of immune-checkpoint inhibitors (ICIs) via the release of different microbial products or metabolites. The microbial-derived STING agonist, cyclic di-AMP (c-di-AMP) can induce monocytes in the tumour microenvironment (TME) to produce type I IFN and skew the polarization of innate immune cells towards an anti-tumorigenic phenotype. The microbial metabolite trimethylamine *N*-oxide (TMAO) can induce pyroptosis in tumour cells and promote the activation of $CD8^+$ T cell-mediated antitumour immunity. TMAO can also promote the induction of tumour-associated macrophages that potentiate IFNγ signalling in the TME. Inosine can promote T helper 1 cell (T_H1) activation via adenosine receptor ($A_{2A}R$) signalling. Cell wall-derived muropeptides can stimulate the immune system in a NOD2-dependent manner and promote the accumulation of $CD8^+$ T cells and pro-inflammatory monocytes in the TME. Cross-reactivity has been demonstrated between $CD8^+$ T cells specific for an epitope expressed by *Bifidobacterium breve* (SVY) or enterococcal bacteriophages (TMP1) and tumour antigens. Short-chain fatty acids (SCFAs) can enhance the memory potential of antigen-primed $CD8^+$ T cells and promote their differentiation into stem-like $Tcf1^+PD1^+CD8^+$ T cells during PD-1 blockade, thus promoting tumour control. 3HB, 3-hydroxybutyrate; T_{reg} cell, regulatory T cell.

between pretreatment microbial features of the gut and the development of severe irAEs[83,86,89,118]. However, as with the associations between the microbiota and response, a similar lack of consensus exists for its associations with irAEs. Larger studies with comprehensive annotation of the development of possible irAEs will be important. The gut microbiota has a critical role in maintaining intestinal homeostasis and barrier function and is therefore particularly implicated in the development of ICI-induced colitis. Furthermore, an overlap has been reported to exist between microbial features associated with improved antitumour efficacy, such as the association between *F. prausnitzii* and the development of colitis in one study[83], whereas data from other studies suggest that similar microbial features are associated with both response and a reduced incidence of severe irAEs, such as *Bifidobacterium* spp.[79,89]. Among patients receiving neoadjuvant ICIs, those without a response who also developed severe toxicities had the lowest level of pretreatment microbial diversity[89]. In line with this observation, many of the taxa associated with response across studies are also typically associated with good general gut health and have frequently been reported to be depleted in those with various autoimmune or inflammatory diseases[69,119–121].

Whether the development of irAEs is associated with the effectiveness of ICIs remains contentious[32,122,123]. The specific type of irAE and the time of onset are both likely to be relevant considerations. For example, vitiligo has been linked with improved outcomes in patients with advanced-stage melanoma receiving ICIs and is associated with cross-reactivity between tumour and self-antigens[124]. By contrast, *Il10*-knockout mice exposed to anti-CTLA4 antibodies have impaired tumour control despite the ICI consistently exacerbating the severity of colitis[79]. In addition to causing serious morbidities, the development of severe irAEs might necessitate the discontinuation of treatment and/or therapeutic immunosuppression. Several studies have indicated that the use of steroids early in the course of treatment is associated with poor outcomes[31,125–127]. Corticosteroids might also alter the composition of the gut microbiota. Dexamethasone has been shown to increase the abundance of *Bifidobacterium* and *Lactobacillus* species in parallel with limiting colonic inflammation in *Il10*-knockout mice[128].

Data published in 2021 indicate that a greater abundance of *Bacteroides intestinalis* is associated with the upregulation of intestinal IL-1β and the development of intestinal toxicities both in mice and in patients with melanoma[86]. Data from another study indicate that TNF is upregulated in the intestines of patients who developed colitis following treatment with ICIs targeting PD-1 or CTLA4 (ref. 129). DSS-induced colitis is exacerbated by the administration of anti-PD-1–anti-CTLA4 antibodies, whereas prophylactic TNF blockade reduces the severity of colitis without compromising the antitumour activity of the ICIs in mouse models[129]. Similarly, experimental data demonstrate that administration of *B. fragilis* promotes tumour control while also ameliorating the histopathological signs of CTLA4-induced colitis[79]. Mechanistically, *Bifidobacterium* species have been shown to confer protection from intestinal inflammation in the specific context of anti-CTLA4 immunotherapy by enhancing the immunosuppressive capacity of T_{reg} via alterations in mitochondrial metabolism[130,131]. Further investigations of the mechanisms by which the gut microbiota shapes the outcomes in patients receiving ICIs is required, but the evidence to date suggests that gut microbes have a central role in influencing immune tone both locally and systemically. This observation is key to determining the immunostimulatory versus immunoregulatory balance that is likely to underpin both response and the development of irAEs. As such, the gut microbiota is a promising therapeutic target of interventions designed to promote responsiveness to ICIs, whilst reducing the incidence of toxicities and/or treating any irAEs.

Leveraging the microbiota in the clinic

Unlike many of the factors that are known to influence the activity of ICIs, the gut microbiota and resultant immune phenotypes are readily amenable to modification. This amenability presents an opportunity to target the microbiota in a way that promotes responsiveness to ICIs and/or reduces the incidence of toxicities. Numerous clinical studies have identified associations between the microbiota and the activity of ICIs, although a lack of consensus exists on the identity of specific taxa linked with response across different cohorts[80–84,86,89]. This lack of agreement across patient cohorts geographically has undermined confidence in the ability to use microbial data to guide clinical management. The generalizability of microbial findings remains a key challenge across the broader microbiome field. This lack of generalizability extends from the large degree of heterogeneity that exists among the microbiota of each individual, which reflects a variety of environmental and host-related factors that shape both microbial composition and functional capacity, including host genetics, diet and previous antibiotic use[132–136]. Such factors contribute to the assembly of gut microbial communities and can influence both the function of microbes and the susceptibility of an individual's microbiota to modification.

It is likely that functional redundancy amongst taxa, whereby different microbes that might be distantly related can perform the same function, contributes to the discordance in results between studies[137,138]. The lack of a consensus on the most relevant taxon-based biomarkers warrants further consideration of how microbial metabolic processes and products modulate antitumour immunity, regardless of the source microbes. The overall state of the microbial ecosystem and resultant microbial metabolites might be much more important than the presence or absence of any specific individual taxon as particular drivers of response and/or toxicities in patients receiving ICIs[139–141]. In addition to functional redundancy, microbes can function in a context-dependent manner, influenced by interspecies competition or cooperation and/or in response to nutrient availability[74,142,143]. The same microbes might therefore have different roles in different dietary or ecological contexts and these could be either beneficial or deleterious for human health. Shared ecosystem features or the maintenance of a gut ecosystem that supports intestinal integrity, including functional properties such as fibre fermentation and mucin turnover, might be underlying common features linked with more favourable outcomes in patients receiving ICIs. For example, many of the taxa linked with responsiveness to ICIs across cohorts are fibre-degrading microbes with an important role in good gut health, and are known to beneficially coexist where cross-feeding interactions between primary fibre degraders such as *Ruminococcus bromii* or *Bifidobacterium* and butyrate producers such as *F. prausnitzii* support optimal fermentation conditions[119,144,145].

Given the syntrophic nature of the gut microbiota, in which the different components are often interdependent, considering the overall assemblage of the microbial community is important. Emerging evidence suggests that despite the large degree of interindividual variation, recurrent patterns in the composition of the gut microbiota exist across human populations. For example, the microbiome of most individuals tends to be dominated by the same key taxa (Ruminococcaceae, *Prevotella* or *Bacteroides*)[146–148]. An individual's gut microbiota typically remains stable over time and this observation suggests that the intrinsic properties of an individual's microbiota will constrain the relationship with certain characteristics

(such as immune tone or disease risk) or how it will be influenced by selective pressures such as diet[146,147,149]. This stability enables the stratification of individuals into microbial clusters or gut microbial community types (sometimes termed 'enterotypes')[146,147]. Some controversy exists surrounding the clustering of the microbiota into discrete classes[147], although population-based stratification that reflects shared microbial compositions at a community level does provide a useful method of accounting for ecological context and the associated functional differences within variable community conformations. For example, microbial communities dominated by Ruminococcaceae, *Prevotella* or *Bacteroides* all have differential saccharolytic, proteolytic and lipolytic capacities[137]. Accordingly, microbial community composition is strongly linked with long-term dietary patterns[150], with *Bacteroides*-dominated microbial communities associated with the consumption of low-fibre diets high in animal fats and protein, and *Prevotella*-dominated communities associated with high-fibre plant-based diets[150,151]. Ruminococcaceae-dominated microbial communities have been suggested to be more functionally redundant and less susceptible to perturbations compared with Bacteroidaceae-dominated communities, which are more frequently associated with enhanced systemic and intestinal inflammation[137,149,152,153]. The overall ecological state of the gut microbiota might therefore be a useful predictor of immunological tone and response to microbiota-targeting interventions.

Cross-cohort analyses of data from patients receiving ICIs have clearly shown that no single microbial taxon alone can be regarded as fully predictive of response or toxicities[87,88]. However, accounting for underlying community assembly between patient cohorts by stratifying patients into groups with shared microbial community level composition demonstrates that differences in microbial ecology underpin the relationship between gut microbes and these clinical outcomes[89]. Of note, patients with Ruminococcaceae-dominated microbiomes, which are linked with higher dietary fibre consumption, are more likely to respond to ICIs[89]. Similarly, in another study, favourable outcomes were associated with the presence of community clusters enriched with Ruminococcaceae[88]. Conversely, patients with microbiomes with a greater relative abundance of microbes that possess the ability to degrade intestinal mucin, as well as patients with increased levels of several systemic inflammatory markers (higher C-reactive protein or neutrophil to lymphocyte ratio) seem to have less favourable outcomes, suggesting the existence of a link between the baseline microbiota and immune fitness[88,89]. Particular ecological states and/or microbial community structures could serve as indicators of risk of non-response and/or toxicities, and enable the identification of patients most likely to benefit from microbiota-related interventions. Substantial evidence now exists that an individual's baseline microbiota influences the success of microbiota-targeting interventions, including dietary interventions, FMT and probiotic engraftment[154–156]. Accounting for the overall assembly of microbial communities is therefore also likely to be important for attempts to reproducibly target the microbiota clinically when attempting to improve the outcomes in patients receiving ICIs.

In order to optimally leverage the gut microbiota in the clinic, more research is required to better understand the functions underpinning the community networks that facilitate responsiveness to ICIs. Clinical tools utilizing the microbiome will need to capture the profound interindividual heterogeneity. Considering the overall assemblage of microbial communities by subclassifying patients based on shared ecosystem features such as into 'community types' provides one potential method for achieving this. Furthermore, different microbial assemblages or ecological states could be utilized as biomarkers of response and/or irAEs[88,89]. Thus, developing tools that can be applied clinically to reliably classify patients into different ecological groups will be important. Further mechanistic interrogation of data from large metagenomic sequence-based datasets in order to identify shared metabolic functions or metabolites rather than individual microbes will also be important to overcome the issues of functional redundancy and interindividual microbial heterogeneity. Metagenomics (shotgun sequencing to sample all genetic material) is a useful research tool that has been used to assess the microbiome at high levels of detail in many studies; however, adapting these methods as a point of care test is not currently feasible owing to the extensive and time-intensive bioinformatics required. Clinical tools that leverage data from 16S rRNA gene amplicon sequencing, which is focused on a specific genomic region only, might be a more feasible method for obtaining results within a turnaround time that permits the guidance of treatment decision-making. This approach is conceptually similar to the use of targeted next-generation sequencing panels as opposed to whole-genome sequencing in other settings, such as testing for targetable genomic alterations in patients with NSCLC and certain other cancers[157,158]. We emphasize that a 'good' microbiota is not the only factor that influences the efficacy of treatment. It is likely that any clinical microbiota-related metrics will need to be combined with assessments of other clinical and/or tumour-intrinsic factors in order to optimize clinical utility. The gut microbiota might have a greater influence on the outcomes in certain patients. Thus, more integrated analyses of the relationships between the gut microbiota and tumour-intrinsic factors will be critical to understanding the role of the gut microbiota during treatment and identifying patients who are most likely to derive benefit from microbiota-targeting interventions.

Strategies for modulating the gut microbiota

Modulating the microbiota to induce an immune–microbiota landscape that is more conducive to a response to ICIs and less prone to the development of severe irAEs is a prominent focus of ongoing research. A variety of strategies including both broad interventions (dietary interventions or FMT) and narrow interventions (specific prebiotic nutrients, probiotic microbes or specific metabolites) might be considered (Box 1). Data from several preclinical studies have demonstrated the capacity to modulate the gut microbiota in a way that improves the activity of ICIs[77–80,82]. However, a caveat relating to a number of these findings is that the microbiota is modulated prior to tumour inoculation, thus altering the baseline microbiota and the immune environment in which the tumour initially develops. This approach is less relevant in the clinical setting, in which modulation of the microbiota would be required after the cancer has evaded an initial antitumour immune response, when a patient might present with advanced-stage and/or metastatic disease. Nonetheless, this disconnect does not preclude the utility of microbial interventions in the clinic and a number of clinical trials are currently underway in this space[159,160]. However, the optimal method for modulating the gut microbiota to promote response to ICIs, including the nature and timing of interventions, requires further investigation, particularly relating to the challenges highlighted above.

A particular microbiota-targeting intervention might result in successful engraftment or modulation of the gut microbiota composition leading to favourable changes in one or more immunological parameters, although these effects might only translate to an improved response to treatment if the tumour is intrinsically capable of responding. Moving

Box 1

Strategies for modulating the gut microbiota to improve the outcome in patients receiving immune-checkpoint inhibitors

- **Diet**
  - Nutritional interventions involving holistic dietary changes such as fibre-rich or ketogenic diets.
  - Interventions can be administered concurrently with ICIs.
  - Advantages include safety, cost-effectiveness, a lack of invasiveness and possible improvements in general health.
  - Disadvantages include difficulties with compliance and changing ingrained dietary patterns, and variable effectiveness dependent on baseline microbiota and diet.
  - Development of precision nutrition tools to design personalized dietary plans based on the baseline microbiota of each patient.
- **Prebiotics**
  - Nutrient supplements that promote the growth of beneficial bacteria already present in the gut, such as inulin or pectin.
  - Advantages include providing a simple, cost-effective and non-invasive method for increasing specific nutrients with the potential to increase the abundance of particular target organisms, potentially with a lower burden for patients, compared with holistic dietary changes.
  - Disadvantages include variable effectiveness depending on the native microbiota, uncertainties regarding the quantities of supplement required to achieve the desired effects, and the possibility that prebiotics from wholefood sources may be more effective.
  - Identification of the optimal prebiotics and intake strategies based on underlying knowledge of their interactions with the patient's diet and/or baseline microbiota is an important step.
- **Probiotics**
  - Preparations of live microbes that are typically administered orally once or twice per day, and can be administered concurrently with ICIs.
  - Advantages include providing a cost-effective, easy-to-use and non-invasive method for introducing beneficial microbes that might not already be present.
  - Disadvantages include variable engraftment uptake depending on the native microbiota, varying levels of ability to survive intestinal transit, and some evidence suggesting that over-the-counter probiotics can reduce microbial diversity and are linked with lower response rates in patients receiving ICIs.
  - Develop next-generation probiotics that comprise several strains of microbes that are known to be consistently associated with a response to ICIs.
- **Postbiotics**
  - Metabolically or biologically active microbial-derived compounds such as metabolites or bacterial cell wall components.
  - Advantages include the ability to bypass the use of microbes and related engraftment issues.
  - Such interventions require a more-detailed mechanistic understanding in order to design and develop effective interventions.
  - Identification of appropriate postbiotics, a next-generation therapeutic approach that utilizes microbial products associated with a response to ICIs.
- **FMT**
  - Transfer of a donor's microbial ecosystem to a recipient either via colonoscopy or as orally administered capsules.
  - FMT is generally administered prior to treatment with ICIs. Maintenance stool-derived capsules are also being assessed in certain scenarios as well as antibiotic pretreatment.
  - Advantages include the transfer of both the putative favourable immunogenic microbes and their diverse supporting ecosystem.
  - Disadvantages include variable levels of engraftment and scalability issues depending on the donor.
  - Requires the identification of optimal donors and strategies for inducing engraftment.
- **Designer consortia**
  - Administration of a defined microbial consortium of specific cultivated species. Daily doses for the period of the trial, with short lead-in periods (4 days to 2 weeks) prior to administration of ICIs are currently being assessed.
  - Advantages include providing an intervention that balances the complexity of FMT with the scalability and practicality of probiotics.
  - Disadvantages include variable levels of engraftment, and the need to identify the optimal microbial strains.
  - Future research directions include developing consortia comprising several strains of microbes known to be consistently associated with a response to ICIs.

FMT, faecal microbiota transplantation; ICI, immune-checkpoint inhibitor.

forward, patient selection will be an important consideration when designing trials assessing the effectiveness of microbiota-targeting interventions that are intended to improve the efficacy of treatment. For example, response rates to subsequent lines of anti-PD-1 antibodies can vary greatly depending on the underlying cause of disease progression (such as primary versus acquired resistance)[161,162]. Furthermore, microbial interventions alone are likely to be effective only in a subset of patients or might need to be administered in combination with other interventions such as novel ICIs in certain scenarios[163].

Diet and prebiotics

Nutritional interventions provide an appealing method for modulating the microbiota, owing to their excellent safety profile, cost effectiveness and non-invasiveness. Holistic dietary changes and/or

supplementation with specific nutrients (prebiotics) could be utilized to expand the population of 'beneficial' microbes and/or shift the immune–microbiota landscape. Dramatic dietary alterations such as from an animal-based diet to a plant-based diet have been shown to rapidly alter the gut microbiota and related metabolites within a short time period (1–4 days)[151,164,165]; however, these changes are also readily reversible, indicating that sustained dietary alterations are likely to be required to achieve long-term health benefits. The ability to rapidly alter the gut microbiota and its metabolism highlights the utility of short-term dietary alterations either prior to receiving, or during treatment with, ICIs as a method of augmenting treatment effectiveness, and/or reducing susceptibility to toxicities.

Consumption of a high-fibre diet (meeting the daily fibre intake of 30 g/day recommended in many guidelines) is associated with a reduced risk of a variety of metabolic and inflammatory diseases as well as cancer[166]. However, a large proportion of individuals worldwide do not meet this dietary recommendation[166]. Consumption of >30 types of plants (fruit, vegetables, grains) per week has also been associated with a healthier and more diverse microbiota[167]. Key fibre-fermenting microbes such as *F. prausnitzii*, and faecal SCFA concentrations, have been observed in individuals without cancer who switch between high-fibre diet and zero-fibre 14-day diets[168]. In patients with cancer, higher fibre intake, greater microbial diversity, and a greater abundance of fibre-fermenting microbes such as *F. prausnitzii* are all associated with a response to ICIs, suggesting that even short-term dietary interventions that increase fibre intake can augment the activity of ICIs[82–85,89]. However, such interventions are more likely to benefit those whose diet did not previously meet the recommended level of fibre intake rather than entire cohorts. In a phase I study involving melanoma survivors, a high-fibre dietary intervention, in which patients received a fibre-enriched wholefood diet for 6 weeks (containing 40–50 g of fibre per day), was found to be feasible. Notably, rapid shifts in both the gut microbiota and the metabolome were observed alongside increases in SCFA-producing taxa as well as SCFA acetate, omega 3/6 and tryptophan metabolism[169,170]. The largest degree of shift in the gut microbiota was observed in patients with the lowest baseline fibre intake, suggesting that the effects of baseline diet and microbiota are an important factor in determining the effectiveness of the intervention. A phase II randomized controlled trial assessing the efficacy of an 11-week dietary intervention comprising a fibre-enriched wholefood diet on the gut microbiota of patients with melanoma receiving ICIs is currently ongoing (NCT04645680).

Dietary supplementation with specific prebiotics might be an alternative strategy to modulate the microbiota; however, this approach might only promote the expansion of specific microbe populations rather than enhance the overall level of microbial diversity. Preliminary evidence from mouse models suggests that the administration of inulin prebiotics can promote antitumour immunity. In one study, supplementation with inulin was found to reduce implanted tumour growth in mouse models of melanoma and overcome resistance to MEK inhibitors[171]. In another study, oral administration of inulin gel was found to enhance the activity of anti-PD-1 antibodies across multiple mouse models[112]. Elsewhere, a pectin-enriched diet has been shown to induce type I IFN production in the TME and to improve both tumour control in the absence of therapy and the activity of ICIs in mouse models[105]. Pectin is known to be metabolized by several microbes associated with responsiveness to ICIs including *F. prausnitzii* and *B. fragilis*, as well as to stimulate mucin production[172]. However, determining whether dietary supplementation with this or similar prebiotics at tolerable doses is effective in patients with cancer requires further investigation.

A ketogenic diet characterized by high levels of fat, moderate levels of protein and few carbohydrates has also been shown to enhance the antitumour activity of anti-PD-1 antibodies in mouse models, via the principal ketone body 3-hydroxybutyrate (3HB)[173]. 3HB mediates these effects by inducing immunostimulatory effects in the spleen, which promote the expansion of $CXCR3^{+}CD8^{+}$ T cells while also restraining the ICI-induced upregulation of PD-L1 on myeloid cells and leading to sustained T cell activity. The ketogenic diet resulted in substantial alterations in the gut microbiota. Nonetheless, the authors proposed that the microbiota does not have a central role in the antitumour effects of the diet as simultaneous administration of antibiotics had no meaningful effects on antitumour activity. Rather, the ketogenic diet appeared to directly stimulate the immune system. This strategy contrasts with the concept of a high-fibre diet conferring improvements in antitumour immunity, although interestingly both 3HB and SCFAs can converge on similar pathways such as the metabolite-sensing receptor hydrocarboxylic acid receptor 2 (GPR109A)[57,173]. Together with data on fibre intake, these findings suggest that several different dietary strategies are possible and might benefit different individuals in terms of augmenting antitumour immunity. A clinical trial assessing the efficacy of a ketogenic diet involving either continuous or intermittent scheduling or β-hydroxybutyrate supplementation in combination with nivolumab and ipilimumab in patients with metastatic RCC is currently underway (NCT05119010).

While diet shapes the composition of the microbiota, how an individual responds to a particular dietary intervention is often dependent on the composition of the individual's baseline microbiota. For example, responses to supplementation with the same fibre-rich diet are often highly heterogeneous[142,145,154,174]. A higher ratio of *Prevotella* to *Bacteroides* is associated with the ability of fibre to improve glucose metabolism in individuals without cancer[154]. In another study, higher faecal butyrate levels were seen in individuals who had detectable *R. bromii*, prior to supplementation with resistant starch obtained from potatoes[145]. Interspecies competition within the microbiota shapes which microbes respond to given fibres (for example, species within the *Bacteroides* genus have overlapping capacities for the metabolism of glycans), and predicting which species will respond to a particular fibre supplementation requires consideration of microbial community context (such as which other *Bacteroides* species are present)[142]. This large degree of interindividual heterogeneity, which creates variable responses that seem to be dependent at least in part on the microbiota assemblage, presents a challenge to the design of effective therapeutic dietary interventions. This observation highlights the importance of a personalized rather than a 'one size fits all' approach that includes the consideration of ecological context and microbial community types. Another consideration is that different forms of dietary fibre have different prebiotic effects. This observation reflects the fact that microbes respond differently to fibre from different sources and that not all fibre is equally proficient at stimulating, for example, SCFA production[145,164]. Therefore, considerations of both the type of dietary fibre and an individual's baseline gut microbial composition are relevant to achieving the desired effects. Encouragingly, precision nutritional tools that integrate data on the microbiota to predict individualized diets, here in the context of augmenting postprandial glycaemic spike in non-diabetic individuals[156], are beginning to be developed.

Antibiotics

Antibiotics are known to reduce microbial diversity and thus alter the composition of the microbiota. The activity of these agents has been extensively employed to study the role of the gut microbiota in a variety of inflammatory diseases and cancer[175,176]. For example, data from several preclinical studies indicate that antibiotics can suppress colon tumorigenesis through the elimination of carcinogenic bacteria[177,178]. Several preclinical studies have also shown that antibiotics can impair antitumour immunity in the context of both chemotherapy and immunotherapy[77,79,119]. In patients with cancer receiving ICIs, the use of antibiotics up to 30 days prior to the commencement of treatment is associated with inferior outcomes[179]. In a prospective cohort of 69 patients with advanced-stage RCC, those who received antibiotics within 2 months prior to treatment with anti-PD-1 antibodies had significantly reduced objective response rates than patients who did not (28% versus 9%; $P < 0.03$)[180]. These patients also had lower levels of microbial diversity. Data from a meta-analysis pooling data from 11,959 patients receiving one or more ICIs across 38 studies highlighted the near-universal deleterious effects of antibiotics in this population, with antibiotic use being associated with increased mortality, particularly when administered prior to treatment initiation[98]. In addition to response, several retrospective studies have found that antibiotic use prior to receiving ICIs is associated with an increased incidence of severe irAEs[181,182]. Data from another study demonstrate that antibiotic use, especially after initiation of treatment with ICIs, is associated with a higher risk of severe colitis[183].

The use of antibiotics as a strategy to modulate the gut microbiota has been explored in certain conditions in an attempt to eradicate harmful or pathogenic bacteria present within the microbiota; however, the non-specific nature of most antibiotics can also deplete beneficial commensals[176]. Overall, antibiotics are unlikely to be a useful strategy for improving the effectiveness of ICIs, and indeed should be avoided wherever possible. In some scenarios, however, antibiotics are unavoidable. Strategies to prevent antibiotic-induced dysbiosis and to limit the deleterious effects of these agents on the efficacy of ICIs are therefore of interest. For example, the administration of DAV132, an orally administered colon-targeting adsorbent, in combination with antibiotics has been shown to preserve microbial diversity[184,185]. Furthermore, the activity of anti-PD-1 antibodies was preserved in germ-free mice that received FMT from human volunteers without cancer who had received antibiotics in combination with DAV132 (ref. 185). This finding might also be relevant in the context of enhancing the engraftment of microbiome-targeting interventions.

Probiotics

Probiotics are preparations of live microbes that are administered in an attempt to improve the microbiota. Dietary interventions, including prebiotics, are largely intended to cultivate microbes that are already present in the gut ecosystem, whereas probiotics have the capacity to introduce new microbes that might not already be present. Typical over-the-counter probiotic formulations contain single strains of readily culturable microbes, such as *Bifidobacterium* and *Lactobacillus* species, which have been associated with certain anti-inflammatory properties in the gut[186]. However, advances in technology might soon enable the development of next-generation probiotics involving microbes that were previously limited by their stringent growth requirements, including strict anaerobes such as *F. prausnitzii* and *A. muciniphila*[187,188]. Probiotics featuring such microbes have shown convincing results in mice, whereby oral administration as adjuncts to single-strain probiotics restored the antitumour activity of ICIs and abrogated the effects of antibiotics and/or a less-favourable microbiota[78,81,105].

The success and reproducibility of probiotics in humans, however, is affected by several factors associated with their composition, dose and ability to survive gastrointestinal transit, as well as their capacity to colonize the gut, in which the engraftment of probiotic strains has been shown to be strongly influenced by the pre-existing microbiota[155]. These considerations might contribute to the often heterogeneous responses to probiotic supplementation and complicate the ability to clinically assess the effectiveness of such interventions[187]. Conflicting evidence exists as to whether currently available probiotics offer any benefit or are even detrimental in the context of cancer[98,159,160,189]. In fact, probiotic use has been shown to impair microbiota reconstitution following administration of antibiotics in at least one study[190]. Over-the-counter probiotics might have detrimental effects on the outcomes in patients with cancer receiving ICIs, and have been associated with both lower levels of microbial diversity and lower response rates[85]. Furthermore, probiotic administration was found to impair tumour control following the administration of an anti-PD-L1 antibody in mice receiving FMT from an ICI-responding patient[191]. Synbiotics, which combine probiotics and prebiotics, might be a more successful alternative to prebiotics in terms of facilitating microbial diversity and overcoming some of the limitations associated with single-strain probiotics. The introduction of precision approaches into the probiotic space is also likely to enhance the reproducibility and efficacy of probiotics in terms of the ability to alter the microbiota, augment immune responses and thus support tumour clearance and/or limit the incidence and/or severity of irAEs. This could enable selection of the optimal probiotic in a way that is tailored to the needs of a specific patient and the patient's microbiota[160,187]. Several clinical trials testing these personalized approaches are currently underway (NCT04699721, NCT03829111 and NCT04601402). In one of these phase I studies, CBM588, a bifidogenic probiotic strain did not substantially change the overall abundance of *Bifidobacterium*. However, an increase was observed in responders, and patients receiving the probiotic in combination with ipilimumab and nivolumab had significantly longer PFS than those receiving ipilimumab plus nivolumab only (12.7 months versus 2.5 months, HR 0.15, 95% CI 0.05–0.47; $P = 0.001$)[192].

FMT

FMT involves the transfer of a donor's microbial ecosystem to a recipient, usually via either colonoscopy or as orally administered capsules. FMT is routinely used to treat recurrent *Clostridium difficile* infections, and has an established safety profile in that setting[193,194]. Two phase I trials have assessed the safety and feasibility of FMT in combination with the reintroduction of an anti-PD-1 antibody in patients with metastatic melanoma who had previously progressed on immunotherapy[195,196]. These studies were not scaled to assess efficacy; nonetheless, preliminary data are promising and indicate that FMT in combination with the reintroduction of an anti-PD-1 antibody is not only safe and feasible but can potentially overcome resistance in a subset of patients. The responses seen across both studies were contingent on successful engraftment and were associated with favourable immune reprogramming both locally in the gut and in the TME[195,196]. Determining whether the lack of response to ICI rechallenge is linked with the engraftment of transplanted material, the circumstances of disease progression and/or tumour-intrinsic factors will be an important step in truly assessing the efficacy of FMT in this setting. As such, any future clinical trials will need to be carefully

designed. Concurrent assessments of the roles of the microbiota and tumour-intrinsic factors will be important to achieving this goal.

FMT engraftment can potentially be enhanced by using antibiotics to clear microbial niches prior to transplantation. Data from the two previously mentioned phase I trials demonstrate this incongruence in the success of FMT engraftment. Administration of antibiotics before treatment, as used in one study[191], resulted in more successful engraftment than in the other study[192] in which competition between donor and recipient microbes in the absence of antibiotics is likely to have influenced the ability of the transplanted material to modulate the microbiota[195,196]. Antibiotics do not appear to reduce the effectiveness of FMT and provide a potential means to reduce the extent of inter-individual engraftment variation enabling the clearer delineation of the mechanisms underpinning efficacy. An evident gap in our understanding of the mechanisms through which FMT modulates immune function continues to exist; nonetheless, the nature of FMT enables the transfer of both the putative favourable immunogenic microbes and their diverse supporting ecosystem. Selection of the most appropriate donors will be crucial to the outcome of future studies. Nonetheless, the 'ideal' microbiota in terms of facilitating a good response to ICIs, and therefore the ideal donor, remains to be defined. This limitation can be partially overcome using donor material from patients with metastatic melanoma who had a durable complete response to an anti-PD-1 antibody. However, such strategies might have limited scalability. Several phase I and II trials seeking to determine the effects of FMT using a variety of donor sources and strategies on the efficacy of ICIs in patients with melanoma and in several other solid tumour types including NSCLC, RCC and prostate cancer in both the treatment-naive and refractory settings have recently been completed or are ongoing (NCT03772899, NCT04951583, NCT04577729, NCT05251389, NCT04758507, NCT04988841, NCT04521075, NCT04116775 and NCT05008861). Preliminary results from a phase I trial involving patients with anti-PD-1 antibody-naive advanced-stage melanoma who underwent FMT derived from a donor without cancer prior to receiving an anti-PD-1 antibody suggest that FMT might ameliorate primary resistance[197]. Phase II trials involving larger cohorts of patients with ICI-naive disease are now being conducted (NCT04951583).

Beyond modulating responsiveness to ICIs, FMT might also have a role in ameliorating susceptibility to toxicities. The initial case series of two patients with ICI-induced colitis who were successfully treated with FMT was reported in 2018 (ref. 198). This reported success supported the feasibility of modulating the gut microbiota to reduce the risk of severe irAEs. Several trials exploring the efficacy of FMT in patients with steroid-refractory ICI-induced colitis have been conducted or are ongoing (NCT03819296, NCT04038619 and NCT04883762). Preliminary data from 37 patients enrolled in these trials indicate a symptom response rate of 83.7% and remission of colitis symptoms in 94.6% within 12 weeks of FMT, highlighting a potential role of FMT in this setting[199]. A trial investigating prophylactic FMT with the aim of reducing the risk of ICI-induced colitis in patients with RCC receiving ipilimumab plus nivolumab is currently ongoing (NCT04163289).

Designer consortia and other microbial strategies

Administration of a defined microbial consortium of cultivated species provides an alternative gut microbiota modulation strategy that balances the benefits of the ecological complexity of FMT with the scalability and practicality of probiotics. Currently, three registered trials (NCT03686202, NCT03817125 and NCT04208958) combining such consortia with ICIs in patients with advanced-stage cancers have been conducted. Preliminary data from one of these trials (NCT03686202) were reported in February 2023 (ref. 200). In this study, patients received MET4, an orally administered microbial ecosystem therapeutic comprising 30 species isolated from a donor without cancer that have previously been associated with responsiveness to ICIs in 40 patients with a range of advanced-stage solid tumours. MET4 was found to increase the relative abundance of MET4 taxa in a subset of patients (increases in more than five MET4 taxa were observed in 35% of recipients), although the extent and number of species changes varied substantially between patients. This intervention was found to be safe and tolerable with evidence of shifts in plasma metabolite levels in patients with successful engraftment[200]. Similar limited levels of engraftment were found in the MCGRAW trial (NCT03817125), which involved the administration of SER-401, a microbiome therapeutic enriched in Ruminococcaceae, to patients with metastatic melanoma. SER-401 administered in combination with an anti-PD-1 antibody was found to be safe and well tolerated, although the trial was terminated early as patients receiving SER-401 had a lower disease control rate (37.5% versus 83.3%). The investigators surmised that this reduction might have been linked with the antibiotic preparative treatment that was included in this trial[201].

An improved understanding of the mechanisms through which FMT can modulate immune function will enable the development of more specific approaches. These approaches might include interventions using postbiotics (metabolically or biologically active microbial-derived compounds) rather than the microbes themselves to achieve the desired effect[159,160]. Such compounds could serve as powerful adjuvants for use with ICIs that also bypass at least some of the limitations associated with microbial heterogeneity. An example of an alternative strategy leveraging microbes to enhance the efficacy of ICIs without specifically targeting the microbiota is provided by the ROSALIE trial (NCT04116658). This study was designed to test a therapeutic vaccine comprising gut microbiota-derived peptides designed to activate commensal-specific memory T cells that are able to cross-react with highly homologous tumour antigens plus nivolumab in patients with glioblastoma[202]. Preliminary data indicate robust immune responses to at least one of the three microbiota-derived peptides in almost all patients, suggesting that this approach might be effective in patients with neoantigen-low tumours[203].

Lessons learned from early trials

1. Microbial engraftment and/or an ecological response is the first key hurdle for any microbiota-targeting therapy. The available data from phase I studies indicate suboptimal levels of engraftment and/or variable responses across all modalities[192,195,196,200,201]. This heterogeneity probably reflects the variations inherent to the native microbiota and diet of each patient. Phase I trials are not scaled to assess efficacy; nonetheless, the available early phase data demonstrate that responses to ICIs in patients who were previously refractory to these agents are contingent on successful engraftment[195,196]. Enhancing or reproducibly predicting successful engraftment will therefore be a key challenge. Results on the use of antibiotics to deplete the native microbiota and thus promote engraftment without impairing the efficacy of ICIs are currently discordant[195,201].
2. The development of interventions that are both scalable and reproducible will be an important step. Trials testing FMT also provide the opportunity to enhance our mechanistic understanding, although a key goal should be to progress towards more specific and reproducible interventions, which might take

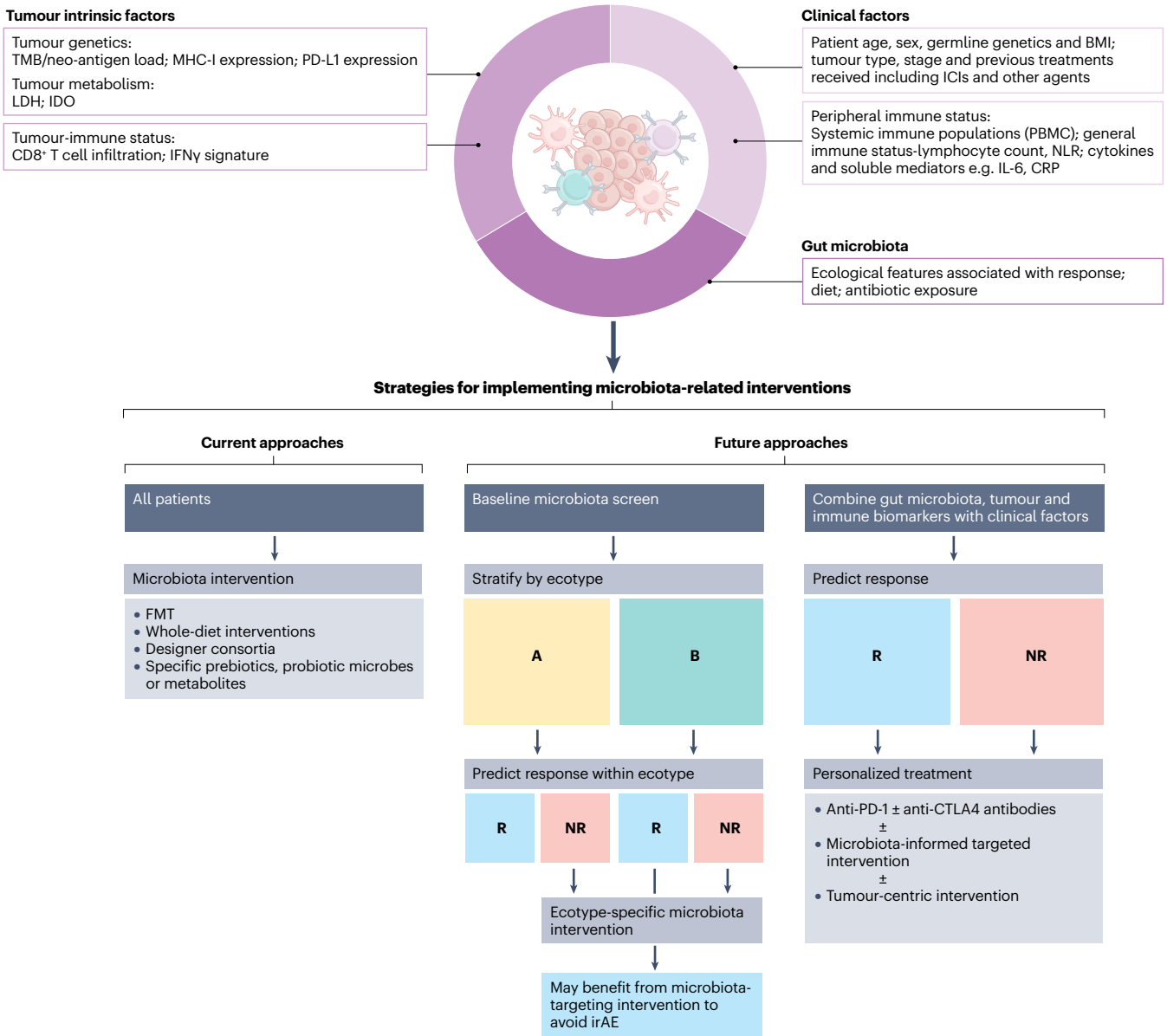

Fig. 3 | Integrating the gut microbiota with immunological and tumour-intrinsic factors for personalized treatment approaches. A variety of factors can influence whether or not a patient will respond to an immune-checkpoint inhibitor (ICI) and/or whether immune-related adverse events (irAEs) are likely to accompany such treatment. These include tumour-intrinsic factors, immunological parameters (intratumoural and systemic), clinical factors and the gut microbiota. Current strategies for assessing interventions targeting the gut microbiota in combination with ICIs involve giving all patients the same intervention irrespective of their baseline gut microbiota or diet. Future approaches should use a patient's baseline gut microbiota to determine the most appropriate targeted intervention. Microbial ecology or community assemblages (ecotype) should also be accounted for in predicting clinical outcomes and in selecting the most appropriate intervention. This approach might progress towards personalized treatment guided by the gut microbiota in combination with other clinical factors and tumour or immune biomarkers. CRP, C-reactive protein; FMT, faecal microbiota transplantation; IDO, indoleamine-2,3-dioxygenase; LDH, lactate dehydrogenase; NLR, neutrophil to lymphocyte ratio; NR, non-response; PBMC, peripheral blood mononuclear cell; R, response; TMB, tumour mutational burden.

the form of defined consortia and/or probiotics, or microbial products and/or metabolites. The identity of the ideal microbiota and the most effective donor strategy are also both currently unclear. A variety of approaches are therefore being assessed, including FMT from donors who had a response to ICIs, from those without cancer or from pooled donors, as well as making comparisons with FMT from patients who do not respond to ICIs to control for possible intervention-related effects.

3. In an ICI-naive cohort, a response to one or more ICIs would be expected in a substantial proportion of patients (typically

around 50% of patients with metastatic melanoma). By contrast, response rates in patients who are rechallenged with anti-PD-1 antibodies following previous disease progression can be very low depending on the circumstances of progression. Patients who previously had a response and had disease progression off-therapy might respond well to rechallenge[162]. Those with primary resistance might respond to the introduction of an additional ICI[204,205], but usually do not respond to rechallenge with an anti-PD-1 antibody only.

4. Clinically homogeneous trial populations, including considerations of previous cancer treatments received, tumour type, location and stage, and ICI regimen, will be particularly important when assessing the efficacy of specific microbial interventions. Previous lines of therapy or associated antibiotic use, for example, might all influence the ecological response to a microbiota-targeting intervention. Furthermore, the optimal microbial profile might differ between tumour types. For example, whether the gut microbiota that best promotes responsiveness to ICIs in patients with melanoma is the same as the one that best promotes responsiveness in patients with NSCLC is currently unclear.
5. Robust complementary analyses of tumour material will be important for linking ecological responsiveness or intervention engraftment with tumour responsiveness. This aspect is particularly relevant given that, even if an intervention can shift the gut microbiota and metabolome towards a more favourable immunological phenotype, this might only translate into enhanced responsiveness to ICIs if the tumour is intrinsically amenable to clearance by the immune system (for example, the tumour does not overexpress or acquire mutations in genes associated with antigen presentation and/or immune exclusion) and might be particularly relevant in patients with acquired resistance to ICIs.
6. Results from the phase I controlled feeding study (NCT04645680) indicate the feasibility of, and compliance with, a high-fibre intervention supported by rapid shifts in the gut microbiota and metabolome[169,170]. However, the outcomes also highlight the difficulty of changing ingrained dietary patterns, with patients often reverting to their previous low-fibre diet followed by the re-emergence of the 'native' microbiota and/or metabolic state upon intervention withdrawal. Nonetheless, this observation does not preclude the potential utility of short-term dietary interventions designed to optimize treatment outcomes; that is, long-term and/or lifetime microbiome–immune homeostasis does not necessarily need to be overcome permanently if temporal, rapid shifts can effectively improve the outcomes in patients receiving ICIs.

Conclusions

The study of the gut microbiota in the context of cancer and cancer treatment is an exciting and rapidly evolving field. Substantial evidence now exists indicating that the microbiota has a pivotal role in shaping immune function and that modulating the gut microbiota can alter and/or reprogramme antitumour immunity. Targeting the gut microbiota therefore provides a powerful tool to overcome resistance to ICIs, to reduce the risks of developing severe toxicities and to prevent clinically significant morbidities. Considerable advances are anticipated in this field over the coming decades with the translation of findings from both preclinical and clinical studies into improvements in clinical practice. However, the microbiota is only one of many factors capable of influencing the efficacy of ICIs and, although modulating the microbiota could have profound effects in a substantial subset of individuals, it is likely that such interventions will not be uniformly effective at enhancing the activity of ICIs. For this purpose, it is likely that a range of complementary approaches will need to be developed. Ultimately, combining microbiota-related metrics with other clinical and tumour-related factors will enable the optimal clinical utilization of the microbiota, both as a biomarker and to harness its potential as a readily modifiable therapeutic target (Fig. 3). Much progress has been made, although many questions remain unanswered. For example, what is the ideal microbiota for enhancing the outcomes in patients receiving ICIs? Which patients will derive the most clinical benefit from manipulation of the microbiota? Can we simultaneously enhance antitumour immunity and limit immune-mediated toxicities? What is the best strategy for modulating the microbiota? Interindividual microbial heterogeneity poses a major challenge both in terms of studying the microbiota across human populations and in the translation of microbial findings into clinical interventions capable of reproducibly modulating the microbiota. However, as the field evolves, and with the careful design of clinical trials, enhanced precision and a more personalized approach tailored towards an individual and their microbiota, improved outcomes can be achieved.

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Author contributions

R.C.S. researched data for the manuscript. All authors made a substantial contribution to discussions of content, writing the manuscript and reviewing and/or editing the manuscript prior to submission.

Competing interests

R.A.S. has acted as a consultant and/or adviser to Amgen, Bristol-Myers Squibb, Evaxion, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, MetaOptima Technology Inc, Merck Sharp & Dohme, Myriad Genetics, NeraCare Inc, Novartis, Provectus Biopharmaceuticals Australia and Qbiotics. G.V.L. has acted as a consultant and/or adviser to Agenus, Amgen, Array Biopharma, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Evaxion, Hexal AG (Sandoz Company), Highlight Therapeutics S.L., Innovent Biologics USA, Merck Sharpe & Dohme, Novartis, OncoSec, PHMR Ltd, Pierre Fabre, Provectus, Qbiotics and Regeneron. The other authors declare no competing interests.

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