Modulating the microbiome to improve therapeutic response in cancer

Jennifer L McQuade, Carrie R Daniel, Beth A Helmink, Jennifer A Wargo

Although novel therapies, including immunotherapy, have dramatically improved outcomes for many patients with cancer, overall outcomes are heterogeneous and existing biomarkers do not reliably predict response. To date, predictors of response to cancer therapy have largely focused on tumour-intrinsic features; however, there is growing evidence that other host factors (eg, host genomics and the microbiome) can substantially affect therapeutic response. The microbiome, which refers to microbiota within a host and their collective genomes, is becoming increasingly recognised for its influence on host immunity, as well as therapeutic responses to cancer treatment. Importantly, microbiota can be modified via several different strategies, affording new angles in cancer treatment to improve outcomes. In this Review, we examine the evidence on the role of the microbiome in cancer and therapeutic response, factors that influence and shape host microbiota, strategies to modulate the microbiome, and present key unanswered questions to be addressed in ongoing and future research.

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Department of Melanoma Medical Oncology (J L McQuade MD), **Department of Epidemiology** (C R Daniel PhD), **Department of Surgical Oncology** (B A Helmink MD, J A Wargo MD), **Department of Genomic Medicine** (J A Wargo), **The University of Texas MD Anderson Cancer Center, Houston, TX, USA**

Correspondence to:
Dr Jennifer A Wargo, Departments of Surgical Oncology and Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
jwargo@mdanderson.org

Introduction

The development of novel therapies such as immune checkpoint inhibitors has resulted in dramatic improvements in the outcomes of many patients with cancer. However, outcomes are heterogeneous, with some patients achieving dramatic durable complete remissions, and others deriving no benefit at all. Beyond tumour-intrinsic features that might predict response and drive resistance, there is increasing evidence that host (ie, patient) factors, including the microbiota, might influence response to therapy.[1–6]

The human microbiota is comprised of complex communities of trillions of microbes that live on and inside humans. These commensal microorganisms have co-evolved with humans to have several functions that benefit human health, including harvesting otherwise inaccessible nutrients from the diet, maintaining integrity of mucosal barriers, and contributing to immune system development and homoeostasis.[7,8]

Our understanding of the microbiome has grown exponentially in the past decade with the development of high-throughput sequencing approaches.[7,8] The most common component of the human microbiome that is sequenced is the small 30S ribosome subunit, which is unique to prokaryotes and has regions that vary greatly between different species of bacteria (16S sequencing). This technique can be used to quantify alpha diversity (the number of distinct species present and whether distinct species are evenly represented) and beta diversity (differences in taxonomic abundance profiles between different samples), as well as differential abundance of specific bacterial taxa. By contrast, whole-genome or metagenomic sequencing involves sequencing the entire genomes of all microbes (including viruses, fungi, protozoa, archaea) in a given sample. Metagenomic sequencing has the added advantage of deeper resolution and allows for imputation of function, but at a substantially higher cost in terms of both time and money; however, as with all omics-based profiling, the cost is decreasing and resolution increasing. As such, some components of the human microbiota, such as viruses and fungi, as well as archaea, protozoa, and other microbes, have been less well studied to date than bacteria. Characterisation of these other components of the human microbiota is an area of deep investigation and it is likely that a growing role for these non-bacterial counterparts will be uncovered in the near future. Nonetheless, complexities exist with the characterisation of such components given the vast diversity of virotypes (and viral genomes) that are present in the microbiome, among other variables. Notably, the composition of these other microbial components might directly or indirectly affect the composition of the gut bacterial components; thus, as the field moves forward, these potential interactions must be taken into consideration.

Although novel sequencing techniques have added substantially to our understanding of the human microbiome, we cannot fully understand function and mechanism from computational analysis alone. There has been renewed interest in the field of culturomics—a high-throughput method of culturing microbial species that were otherwise previously deemed difficult or impossible to culture. Culturing bacteria in this way will enable us to study the bacteria themselves rather than their genomes only and thus will help elucidate certain mechanisms of the microbiome in a way that using computational methods alone will not.[9,10]

As our understanding of the microbiota grows, it is becoming increasing clear that the microbiota plays a key role in human health and disease. Disruption of the gut microbiome (dysbiosis) has been implicated in a range of human diseases, including gastrointestinal, autoimmune, neurological, and metabolic diseases.[8] For cancer, specific bacterial and viral infections have been implicated in carcinogenesis[11–16] and have also been associated with treatment-related toxicity to cancer therapy.[3,17–19] Importantly, microbiota (specifically within the gut) have been shown to affect immune responses, with studies reporting strong associations between gut microbiota and response to immune checkpoint

	Patient population	Patients (n)	Intervention	Outcome	Result	Status (location)
Immunotherapy						
NCT03353402	Metastatic melanoma patients resistant to CPI	40	Single-arm: FMT from CPI responders via colonoscopy followed by stool capsules	Engraftment and safety; immune profile change	..	Recruiting (Sheba, Israel)
NCT03341143	Metastatic melanoma patients resistant to CPI	20	Single-arm: FMT from anti-PD1 responders via colonoscopy plus anti-PD1	ORR; immune profile change	..	Recruiting (University of Pittsburgh Medical Center, USA)
NCT03637803	Patients with advanced malignancies resistant to anti-PD1	132	Single-arm: MRx0518 plus anti-PD1	Safety; ORR; immune profile change; microbiome; survival	..	Recruiting (MD Anderson Cancer Center, USA)
Stem cell transplant (SCT)						
NCT03057054	Paediatric allogeneic SCT	500	RCT: *Lactobacillus plantarum* probiotic *vs* placebo	Incidence of GVHD (gastrointestinal primary, other secondary); incidence of blood stream and *Clostridium difficile* infections; change in microbiota; inflammatory cytokines	..	Recruiting (Children's Oncology Group, USA)
NCT00946283	Allogeneic SCT	30	Single-arm: Culturelle (*Lactobacillus rhamnosus GG*)	Safety	..	Terminated (Rutgers University, USA)
NCT03359980	Refractory gut GVHD	32	Single-arm: healthy donor FMT	Gut GVHD; safety culture of multidrug resistant bacteria	..	Recruiting (France)
NCT03214289	Refractory gut GVHD	4	Single-arm: healthy donor FMT via frozen stool capsules	Safety; gut GVHD	..	Recruiting (Sheba, Israel)
NCT03549676	Refractory gut GVHD	15	Single-arm: healthy donor FMT via NJ tube	Gut GVHD; safety; blood biomarkers, infections	..	Not yet recruiting (China)
NCT03492502	Refractory gut GVHD	70	Single-arm: autologous FMT from pre-SCT	Safety; gut GVHD; change in microbiota composition	..	Not yet recruiting (Ramban, Israel)
NCT03720392	Allogeneic SCT	48	RCT: FMT from healthy donor via oral capsules *vs* placebo capsules	Gut microbiome diversity at 1 month after SCT as measured by urinary 3-indoxyl sulfate levels; incidence of GVHD; PFS, OS; infection incidence	..	Not yet recruiting (Massachusetts General Hospital, USA)
NCT03678493	Patients with acute myeloid leukaemia undergoing intensive chemotherapy or SCT	120	RCT: FMT via capsule *vs* placebo capsules	Incidence of infections; FMT engraftment; GVHD	..	Not yet recruiting (University of Minnesota, USA)
Chemotherapy/targeted therapy						
NCT02928523	Patients with acute myeloid leukaemia undergoing induction chemotherapy	20	Single-arm: autologous FMT from pre-chemotherapy	Gut microbiota diversity; culture of multidrug resistant bacteria	..	Completed (France)
NCT02771470	Patients with lung cancer initiating chemotherapy	41	RCT: *Clostrium butyricum* probiotic *vs* placebo	Between group change in microbiome composition, adverse events, immune markers	..	Completed (China)
NCT03314688	Patients with stage I-III breast cancer initiating chemotherapy	250	RCT: ACS recommended diet plus exercise guidelines intervention *vs* usual care	Primary- treatment adherence; secondary- biomarkers including microbiome	..	Recruiting (Yale University, USA)
NCT01410955	Patients with colorectal cancer initiating irinotecan	46	RCT: colon Dophilus probiotic *vs* placebo	Incidence of diarrhoea	..	Completed (Slovakia)
NCT02944617	Patients with metastatic kidney cancer initiating TKIs	20	RCT: Activia yogurt (*Bifidobacterium lactis*) *vs* usual care	Change in levels of *Bifidobacterim*; incidence of diarrhoea; immune markers	..	Recruiting (City of Hope, USA)
NCT02819960	Patients with colorectal cancer initiating irinotecan	100	RCT: PROBIO-FIX INUM probiotic *vs* placebo	Incidence of diarrhoea	..	Completed (Slovakia)
NCT00197873	Patients with colorectal cancer initiating capecitabine, oxaliplatin and bevacizumab	84	RCT: *Lactobacilli* (GefilusR) *vs* placebo	Incidence of diarrhoea; response rate	..	Active but not recruiting (Helsinki University, Finland)
NCT03642548	Patients with NSCLC initiating platinum-based double chemotherapy	382	RCT: Bifico *vs* placebo	PFS; ORR; OS; toxicity		Not yet recruiting (China)
NCT03705442	Patients with metastatic colorectal cancer treated with FOLFIRI	76	RCT: Omni-Biotic 10 *vs* placebo	Grade III or IV diarrhoea; serum zonulin and vitamin D; QOL		Recruiting (University Hospital Rijeka, Croatia)

(Table 1 continues on next page)

	Patient population	Patients (n)	Intervention	Outcome	Result	Status (location)
(Continued from previous page)						
Radiation						
NCT01839721	Patients initiating pelvic radiation	246	RCT: BIFILACT probiotic (*Lactobacillus acidophilus* plus *Bifidobacterium longum*) *vs* placebo	Incidence of diarrhoea; treatment interruption	No difference in overall G2-4 diarrhoea; higher proportion in probiotic group with no G2-4 diarrhoea by EOS	Completed (Canada[27])
NCT01549782	Patients with gynaecological cancer receiving abdominal radiotherapy	31	RCT: inulin and FOS fibre prebiotic (6 g daily) *vs* placebo	Between group change in *Lactobacillus* and *Bifidobacterium*	Higher *Lactobacillus* and *Bifidobacterium* 3 weeks after radiation in prebiotic group	Completed (Spain[28])
NCT01706393	Patients receiving abdomino-pelvic radiotherapy	26	RCT: probiotic (6 strains) *vs* placebo	Gut microbiome composition; diarrhoea, gastrointestinal symptoms	..	Unknown (South Korea)
NCT03420443	Patients with rectal cancer receiving radiotherapy	30	RCT: high fibre supplement plus *Lactobacillus plantarum* *vs* high fibre supplement alone *vs* no supplement	Gut microbiome diversity and gastrointestinal mucosal inflammation	..	Completed (Sweden)
NCT02079662	Patients with stage I-III breast cancer initiating radiotherapy	160	RCT: comprehensive lifestyle intervention (diet, exercise, mind–body) *vs* usual care	Disease-free survival; patient-reported outcomes; microbiome	..	Recruiting (MD Anderson Cancer Center, USA)
NCT01790035	Patients undergoing abdominopelvic radiotherapy	23	RCT: LGG probiotic or placebo	Incidence of diarrhoea; safety	..	Active, not recruiting (Washington University in St Louis, USA)
NCT02351089	Patients with gynaecological cancer receiving radiotherapy	200	RCT: probiotic vs placebo	Incidence of diarrhoea	..	Recruiting (Sweden)
NCT01579591	Patients with rectal cancer receiving chemoradiation	160	RCT: VSL#3 probiotic *vs* placebo	Pathological and clinical response rate; toxicity	..	Unknown (Catholic University of Sacred Heart, Italy)
NCT03742596	Patients with colorectal cancer receiving radiotherapy	40	RCT: probiotic- *Lactobacillus* (eight species) and *Bifidobacterium* (five species) *vs* placebo	Serum immunoglobulins and cytokines; QOL; gastrointestinal toxicity	..	Recruiting (University of Jordan, Jordan)
Perioperative						
NCT00936572	Patients with colorectal cancer in the perioperative period	31	RCT: *Bifidobacterium longum* and *Lactobacillus johnsonii* *vs* placebo	Intestinal colonisation with probiotic bacteria at time of surgery	Dose-dependent colonization with *Lactobacillus* but no colonization with *Bifidobacterium*	Completed (Italy[29])
NCT03072641	Patients with colorectal cancer in the perioperative period	15	RCT: *Bifidobacterium lactis* and *Lactobacillus acidophilus* probiotic plus inulin *vs* no synbiotic	Change in microbiome composition from colonoscopy to surgery	Increased butyrate-producing bacteria (*Faecalibacterium and Clostridiales*)	Completed (University Hospital Gothenburg, Sweden[30])
NCT03358511	Patients who are post-menopausal with stage I-III breast cancer before surgery	20	Single-arm: Primal Defense Ultra Probiotic (13 bacteria)	Change in CD8 T cells from baseline	..	Recruiting (Mayo Clinic, USA)
NCT01895530	Patients with colorectal cancer in the preoperative period	33	RCT: *Saccharomyces boulardii* probiotic *vs* usual care	Colonic mucosal cytokine gene expression	..	Completed (Federal University of Minas Gerais, Brazil)
NCT01479907	Patients with colorectal cancer in the postoperative period	100	RCT: Synbiotic Forte (multistrain bacteria plus fibre) *vs* fibre alone	Gastrointestinal function; QOL	..	Completed (University of Athens, Greece)
NCT01468779	Patients with ampullary cancer patients in the perioperative period	58	RCT: probiotic *vs* placebo	Mortality; infection rate	..	Completed (Hospital de Clinicas de Porto Alegre, Brazil)
NCT02021253	Patients with hepatocellular carcinoma in the perioperative period	64	RCT: probiotic (Lactibiane Tolerance) *vs* placebo	Serum endotoxin levels; inflammatory cytokines; liver function tests	..	Completed (University Hospital Rouen, France)

(Table 1 continues on next page)

	Patient population	Patients (n)	Intervention	Outcome	Result	Status (location)
(Continued from previous page)						
Post-treatment						
NCT01929122	Overweight or obese colorectal cancer survivors	29	RCT: heat-stabilised rice bran, cooked navy bean powder, or placebo	Change in gut microbiome composition and metabolome	Heat-stabilised rice bran and cooked navy bean powder increased gut bacterial diversity and changed composition	Completed (Colorado State University, USA[31])
NCT02843425	Overweight or obese colorectal cancer survivors	60	RCT: usual diet with or without canned navy beans; 8-week cross-over	Change in gut microbiome composition and metabolome	..	Recruiting (MD Anderson Cancer Center, USA)

ACS=American Cancer Society. BSI=blood stream infection. CPI=checkpoint inhibitor. EOS=end of study. FMT=faecal microbiota transplant. FOS=fructooligosaccharide. GVHD=graft-versus-host disease. NJ=nasojejunal. NSCLC=non-small-cell lung cancer. ORR=overall response rate. OS=overall survival. PFS=progression-free survival. QOL=quality of life; RCT=randomised controlled trial. SCT=stem cell transplant. TKI=tyrosine kinase inhibitor.

Table 1: **Trials of gut microbiome modulation in cancer**

blockade and other therapies in human cohorts and murine models.[1,2,4,5,20–23] There is also evidence from preclinical models that successful modulation of the gut microbiota can enhance therapeutic response.

Accordingly, strategies to modulate the microbiome are being used and developed for various human diseases, including cancer. Such strategies include the use of faecal microbiota transplant, which is a safe and effective approved therapy for recurrent *Clostridium difficile*,[24] and is being used experimentally to treat inflammatory bowel disease,[25] metabolic diseases,[26] and even cancer (table 1). Additional strategies to manipulate the microbiome are also under investigation (including probiotic administration and dietary intervention) in multiple diseases, although vast heterogeneity in study design presents a challenge in interpreting the success of these approaches.

In this Review, we assess the evidence for the role of the microbiota in the therapeutic response of cancer, outline the determinants of the microbiota and potential strategies and considerations for microbiota modulation, as well as highlight the complexities with this approach, and a potential path forward for cancer treatment.

The role of microbiota in carcinogenesis

Microbiota have long been implicated in tumour development, with bacterial and viral infections affecting multiple cellular processes (such as metabolism and immune function), with the potential to contribute to carcinogenesis (figure 1). There is certainly evidence for this involvement in the case of luminal gastrointestinal system malignancies, in which bacteria have been shown to contribute to the development of gastric (*Helicobacter pylori*)[11] and colorectal cancers (*Fusobacterium nucleatum*).[12] These specific bacteria have direct effects on the luminal mucosa through several different mechanisms. *H pylori* has been shown to affect processes influencing gastric carcinogenesis via cytotoxins that disrupt autophagy and apoptosis pathways, and modulate key oncogenic signalling pathways such as the Ras/MEK/ERK and β-catenin pathways.[11] *H pylori* also induces chronic inflammatory changes through nuclear factor kappa B signalling and production of interleukin 8.[15] *F nucleatum*, which has been strongly implicated in colon cancer, directly adheres to and invades colonic epithelial cells via the fadA surface protein, which interacts with E-cadherin to mediate changes in β-catenin and Wnt signalling, thereby inducing inflammatory changes and contributing to carcinogenesis.[14]

Data from a large US prospective cohort study further shows that the presence of *F nucleatum* in colorectal carcinoma tissue mediates associations with diet and gradually decreases from caecum to rectum, shedding light on molecular and prognostic differences in colorectal cancer sidedness.[32–34] *F nucleatum*-positive tumours were also associated with a reduced adaptive immune response and shortened survival.[12,35] These and other data have important implications for the widespread use of the faecal microbiome as a surrogate for the gut microbiome. A study,[36] which profiled the mucosal, luminal, and faecal microbiome of healthy individuals and mice along the entire gastrointestinal tract, showed substantial compositional differences at different anatomical locations and between the mucosa, lumen, and faeces. As the microbiota in cancer and normal tissues vary with anatomical location, so might the effects of the microbiota in different anatomical locations on cancer therapy.

Other than the direct effects of specific microbiota on local tissues, there is evidence that the wider community of commensal gut bacteria might modulate cancer risk and progression through competitive exclusion and other mechanisms.[37] Microbial metabolites such as short-chain fatty acids, produced during the colonic fermentation of otherwise indigestible carbohydrates (fibres or resistant starches), play a major role in maintaining intestinal homoeostasis and overall gut health. These microbial products suppress the growth of Gram-negative pathogens, function as energy sources for colonocytes as well as other bacteria (cross-feeding),

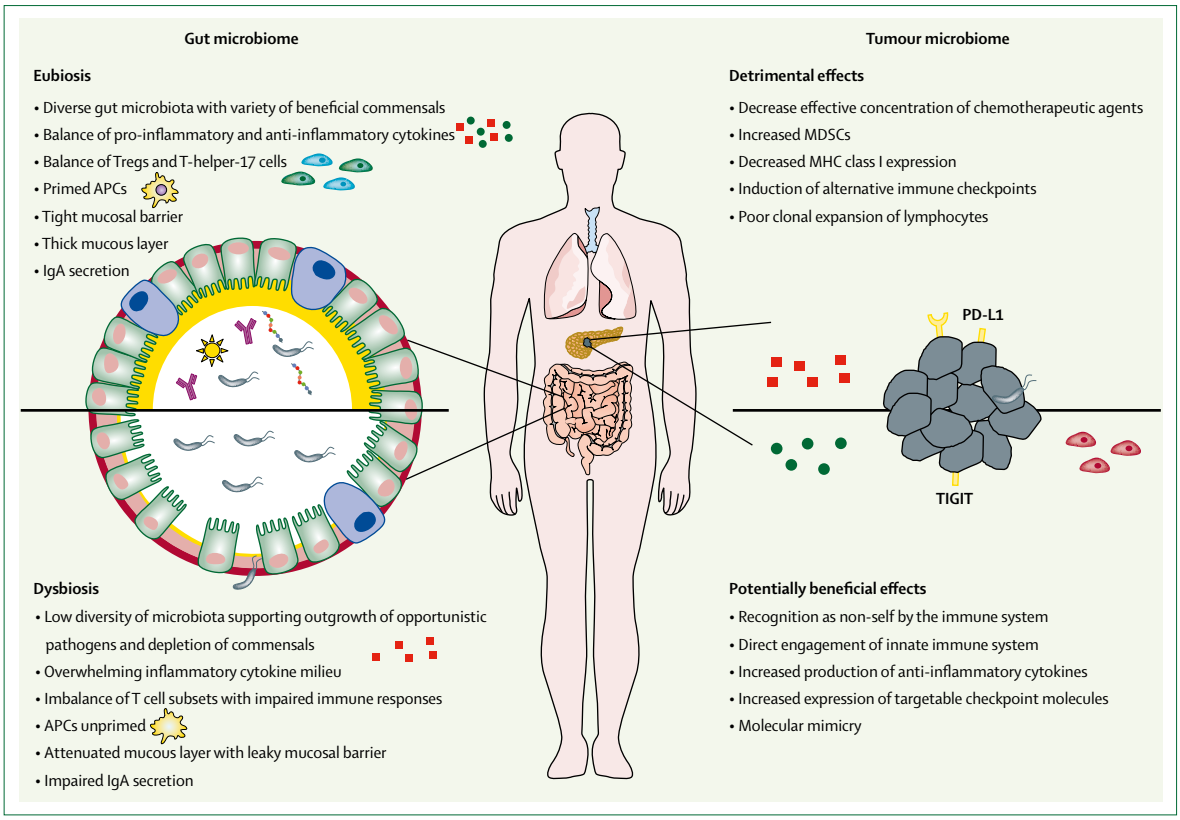

Figure 1: **Complex interplay of the gut and tumour microbiome and the host immune system**
APCs=antigen-presenting cells. IgA=immunoglobulin A. MHC=major histocompatibility complex. Tregs=regulatory T cells. MDSCs=myeloid-derived suppressor cells. PD-L1=programmed death ligand 1.

dampen inflammation, and promote apoptosis of cancer cells.[38] As such, bacteria involved in the biosynthesis and metabolism of short-chain fatty acids are actively involved in maintaining a stable and healthy gut community. Lower abundance of beneficial bacteria that produce short-chain fatty acids have been consistently observed across studies of colorectal cancer and experiments in murine models effectively show that dietary fibre protects against colorectal tumorigenesis in a microbiota-dependent and butyrate-dependent manner.[39,40]

Conversely, secondary bile acids produced by gut microbial enzymes from primary bile acids synthesised by the liver are increasingly recognised as cancer-promoting metabolites. Research in animal and experimental models suggests that the gut microbiome uses bile acids as a messenger to alter the immune function and influence antitumour immunosurveillance.[41,42]

Studies have also highlighted a role for intratumoural bacteria in malignancies such as pancreatic cancer, with evidence that bacteria might be found in tumours of most patients with pancreatic cancer, and can contribute to therapeutic resistance (figure 1).[13,43] Several mechanisms through which intratumoural bacteria might negatively affect treatment response in pancreatic cancer have been described. These effects include the breakdown of chemotherapy into inactive metabolites via bacterial enzymes, leading to treatment resistance,[43] and the induction of myeloid-derived suppressor cells in the tumour microenvironment, and impairing of the response to immune checkpoint blockade,[13] in preclinical models.

Viruses have also been shown to contribute to cancer development in various histologies including cervical cancer, hepatocellular carcinoma, lymphoma, and Merkel cell carcinoma.[16] Studies suggest associations between enteric fungal and viral components and colorectal cancer, though causality has not been shown, and it is not yet clear whether these are direct effects versus more indirect effects on other components of the gut microbiota.[44,45] In this regard, the most prevalent component of the gut virome are bacteriophages, which infect bacteria and can directly affect the bacterial composition of the gut microbiota.

Viruses contribute to carcinogenesis via several different mechanisms. Viruses might induce carcinogenesis indirectly via the initiation of chronic inflammatory states or via global suppression of the immune system, thus affecting anti-tumour immunity (eg, HIV, hepatitis C virus, hepatitis B virus).[16] They might also induce carcinogenesis more directly through viral oncogenes via

direct integration of genetic information into the host genome and the expression of genes that can promote cellular growth and proliferation and induce aberrant DNA damage response pathways (eg, Epstein-Barr virus).[16]

Importantly, virally-driven tumours have been shown to have increased sensitivity to some forms of treatment, such as immune checkpoint blockade, through recognition of non-self (ie, foreign) antigens (figure 1).[46,47] Responses to radiotherapy might be increased secondary to virally-induced DNA damage.[10,48] Responses to other forms of therapy might be related to recognition of viral elements in tumours. T-cell-directed therapies are also being developed to target virally infected tumours.[49] Viruses can negatively alter tumour immunogenicity and the local tumour immune microenvironment as well, reducing class I expression in infected cells or upregulation of immunosuppressive molecules or pathways (eg, programmed death ligand 1) or IDO-1 via induction of an interferon response.[10,50,51]

Vaccination strategies exist to help prevent virally-driven cancers, such as human papillomavirus and hepatitis B, with proven efficacy, although challenges remain to achieve widespread adoption of these vaccines. Viral elements are being increasingly appreciated as tumours are more deeply profiled,[52] and it is likely that effective strategies to target these viruses in cancer treatment and prevention will be developed in the near future.

The influence of gut microbiota on immunity and response to cancer therapy

Although the immune system evolved to fight invading pathogens, a delicate balance exists in the gut–immune axis between tolerance to critical commensal gut microbiota and food antigens and defence against pathogenic microbiota within the gut lumen. Although our understanding remains somewhat lacking, increasing evidence for numerous mechanisms through which gut microbes might influence immunity, both locally and systemically, are being derived (figure 1), with details regarding mechanistic insights based on several key studies.[10,20,21,53–57] Locally, the gut microbiome is essential for preserving the integrity of the mucosal barrier and preventing gut leakiness, which can allow entry of pathogenic or normally commensal bacteria into the bloodstream, activate pattern recognition receptors at distant sites, and elicit immune responses, or inflammation.[54,58] Microbiota within the gut and their metabolites, such as short chain fatty acids (eg, butyrate), might skew the balance of anti-inflammatory and pro-inflammatory cytokines, locally and systemically, and disrupt ratios of regulatory T cells and T-helper-17 cell subsets.[58,59] Gut microbiota might prime local phagocytic cells via tonic signalling to produce cytokines more efficiently in response to an infectious challenge.[60] The implications of dysbiosis for normal systemic immune function include increased susceptibility to certain infections and altered responses to vaccines.[61] Finally, there is increasing evidence that dysbiosis can affect local and systemic anti-tumour immunity in a similar manner; recurrent antibiotic exposure, for example, might be associated with increased cancer risk.[55]

Checkpoint blockade

Landmark studies[20,21] from two independent groups showed that the commensal gut microbiota influenced response to checkpoint inhibition in murine models and also provided evidence that therapeutic response could be enhanced via modulation of the gut microbiota. The translational relevance of these findings to humans was then shown in other studies.[1–5] These clinical cohorts showed a strong association between the composition of gut microbiota and therapeutic response to immune checkpoint blockade across cancer types; however, specific bacterial taxa associated with response varied across the cohorts.

In addition to the associations of gut microbiota with response, two independent cohorts investigating the effects of antibiotic use among patients treated with anti-programmed death receptor-1 (PD-1) inhibitors provide evidence that disrupting the gut microbiota has a deleterious effect on response to checkpoint blockade.[1,62] Lower microbial diversity and altered composition was observed in patients who received antibiotics during treatment with immune checkpoint blockade, suggesting that dysbiosis induced by antibiotic use might impair response to anti-PD-1 therapy. However, not all studies have observed an association between antibiotic use and response to anti-PD-1,[63] and these correlative studies might be confounded by the health status necessitating antibiotic use.[5]

Importantly, mechanistic studies were also done in several of these cohorts, showing that germ-free mice with tumours who received faecal transplants from patients who responded to anti-PD-1 had showed improved response to anti-PD-1 therapy compared with mice that received faecal microbiota transplants from non-responding human patients.[2,4] Patients with a more favourable gut microbiome, as well as mice that received responder transplants showed increased intratumoural immune infiltrates.[1,2,4]

In addition to the gut microbiome's role in response to immune checkpoint blockade, preliminary studies have also shown an association between the gut microbiome and treatment-related immune toxicity.[3,17] These early but important studies focused mainly on colitis, which is a frequent immune-related adverse event, with anti-CTLA-4 therapy, and less frequent but not uncommon with anti-PD1. In these cohorts, baseline differences in gut microbiota were shown to be associated with the development of colitis with anti-CTLA-4 in multiple cohorts; although, as with response, the specific bacteria identified varied among cohorts.[3,17]

Stem-cell transplant

Possibly the earliest work on the role of the microbiota in cancer therapy was done in patients who had allogeneic haemopoietic stem cell transplant (HSCT).[19,64] Conditioning regimens and broad-spectrum antibiotic use can lead to profound dysbiosis in this population. Reduced gut microbiota diversity and loss of specific beneficial bacteria (eg, *Faecalibacterium, Ruminococcus, Lactobacillus,* and *Blautia*) that maintain gut integrity have been associated with transplant-related mortality and graft-versus-host-disease (GVHD).[18,19,65] Disruption of normal commensals can allow for a breakdown in the normal gut integrity and an outgrowth of pathogenic strains. These changes can alter immune homoeostasis, with immune stimulation leading to alloactivation, prompting GVHD and gut bacteria translocation, leading to systemic infections. Although observational studies are unable to dissect the complex interplay in these profoundly immunosuppressed patients between microbiota and overall host health status, infectious complications, altered nutrition, and mortality, animal experiments support a causal role for the gut microbiome in the pathogenesis of GVHD as well as a promising role for microbiota modulation in the prevention and treatment of GVHD.[58,66–68] Data further suggest that viral components of the gut microbiota affect toxicity of cancer therapies, with the presence of a double-stranded RNA *Picobirnaviridae* species predictive of severe gut GVHD following HSCT.[69] In observational patient cohorts, specific gut bacteria (*Eubacterium limosum*) have also been associated with a lower risk of relapse and death after HSCT.[70] Importantly, clinical trials to modulate the microbiota in the HSCT population are underway (NCT03057054, NCT00946283, NCT03359980, NCT03549676, NCT03492502, NCT03214289; table 1).

Chemotherapy

The immune response is a key component of anti-cancer activity of certain chemotherapeutics, and is one mechanism by which the microbiome can affect response to chemotherapy.[22,23] Cyclophosphamide induces the translocation of Gram-positive bacteria such as *Enterococcus hirae* and *Lactobacillus johnsonii* into secondary lymphoid organs, which stimulate Th17 and Th1 immune responses (figure 1).[22] *F nucleatum*, which plays a role in the pathogenesis of colorectal cancer, can also mediate resistance to therapy in this disease. *F nucleatum* targets *TLR4* and *MYD88* innate immune signalling and certain microRNAs, thus activating the autophagy pathway and altering response to chemotherapy.[71] Preclinical models have also suggested that the microbiome contributes to anti-cancer activity of oxaliplatin and to the drug's hyperalgesia toxicity, via induction of reactive oxygen species and pro-inflammatory cytokines.[72]

In addition to the effects of gut microbiota on response to chemotherapy, chemotherapy itself might profoundly affect the diversity and composition of microbiota within the gut.[73,74] A paired cohort study of stool samples from patients before and after myeloablative chemotherapy (who did not receive antibiotics) showed severe compositional as well as functional imbalances with decreased Firmicutes and Actinobacteria and increased Proteobacteria, and altered metabolism.[74] A provocative pilot, placebo-controlled trial of probiotics in paediatric patients undergoing intensive chemotherapy reported reduced rates of systemic infection with probiotics, although this finding needs to be validated and should only be done in the context of a clinical trial.[75]

Gut microbiota might also contribute to the toxicity of chemotherapy and might in turn be targeted. For example, diarrhoea is a common dose-limiting toxicity of irinotecan, the topoisomerase I inhibitor, and is mediated by commensal bacterial β-glucoronidases reactivating the drug into its active metabolite (SN-38) in the gut. Selective enzyme inhibition might be able to preserve the microbiota while protecting against irinotecan-induced toxicity.[76] Trials are currently underway to assess the effects of probiotic supplementation on irinotecan-induced diarrhoea (NCT01410955, NCT02819960, NCT00197873; table 1).

Other therapies

In addition to the effects of microbiota on chemotherapy and immune checkpoint blockade, there is emerging evidence that the microbiota might play a role in response and toxicity to other cancer therapies, including adoptive cell therapy (ACT) and radiotherapy. ACT is a form of immunotherapy whereby activated immune cells (T cells or natural killer cells) are transferred into the host to directly attack the tumour. To date, the relationship between the microbiome and response to ACT in solid tumours in humans has not been reported; however, preclinical models have shown that modulation of the gut microbiota in the setting of treatment with ACT helped to sustain transferred T cells in an IL-12-dependent manner.[77] Preliminary data in clinical cohorts also support the gut microbiome's role in response to radiotherapy, with associations observed between both the gut and cervical microbiome, immune profile, and response to chemoradiotherapy in cervical cancer.[78]

In addition to modulating response, there is also evidence for a contribution of gut microbiota to toxicity from radiotherapy. Multiple preclinical studies and clinical cohorts have shown that radiation-related mucosal injury is associated with marked changes in the microbiome and cytokine signalling.[79] Moreover, germ-free mice are resistant to lethal radiation enteritis, suggesting a functional role for radiation-induced microbiome alterations.[80] Microbiome modulation with faecal microbiota transplant has been shown in preclinical models to alleviate gut radiation injury, or render non-radiated mice susceptible to chemical colitis and radiation injury.[81,82] Early trials of probiotics to prevent radiation-induced mucositis have been promising,

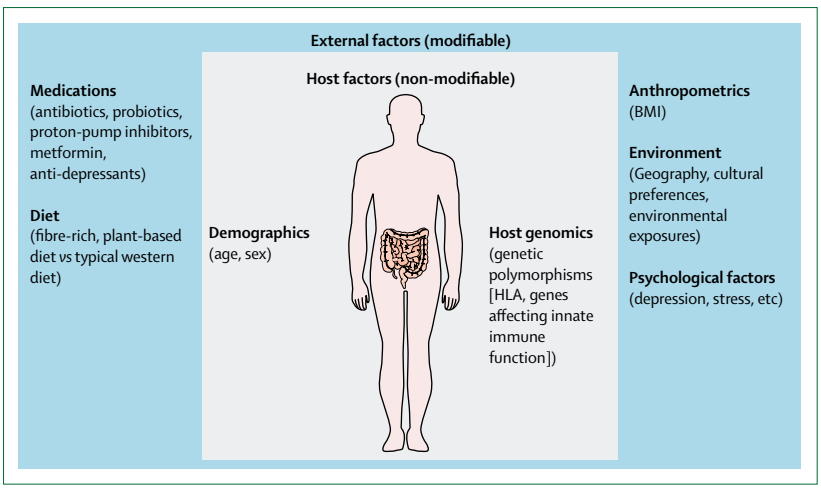

Figure 2: **Factors affecting the gut microbiome**
HLA= human leukocyte antigen.

although more research is needed and heterogeneity between probiotic formulations and dose in these trials is large, limiting generalisability.[83] There are multiple ongoing trials assessing the effect of probiotics (NCT01790035, NCT02351089, NCT01579591) and synbiotics (fibre and probiotics) (NCT03420443) to prevent radiation-induced gastrointestinal toxicity (table 1).[27,28]

Finally, the gut microbiota and other niches, such as the skin microbiota, might be disrupted in the perioperative period. The use of a bowel preparation to limit potential consequences of anastomotic leak, the administration of prophylactic preoperative antibiotics, skin preparation to prevent surgical site infections, perioperative dietary changes (preoperative fasting and postoperative gradual return to normal diet), and the potential for altered bowel motility (ileus) might lead to decreased bacterial diversity and outgrowth of potentially pathogenic bacteria.[84] These effects have important implications for all surgical interventions but require additional consideration for oncologic resections, given the mountaing evidence for the role of microbiota in cancer progression and therapeutic efficacy. This observation has led to several studies examining perioperative probiotics (NCT00936572, NCT03358511, NCT01895530, NCT01468779, NCT02021253) and synbiotics (NCT03072641, NCT01479907) in patients undergoing surgical resection of cancer.

Microbiome as a biomarker in cancer therapy

Increasing evidence from preclinical models and human cohorts has shown that the diversity and composition of gut microbiota are associated with the therapeutic success of different forms of cancer therapy.[1–4,20–22,71,77,78] Along with providing evidence to support therapeutic targeting of the microbiome, these data also substantiate the potential use of gut microbiota as a biomarker of response to cancer therapy. Importantly, this evidence should be considered alongside other predictive biomarkers, including tumour mutational load and other determinants.

Perhaps the most compelling evidence to date with regards to the potential use of gut microbiota signature for response was reported in a cohort of patients with melanoma who were treated with anti-PD1 therapy. This study[2] showed separation of patients into two distinct clusters based on their inherent gut microbiota structure at baseline, on unsupervised hierarchical clustering. Interestingly, one of these clusters was comprised exclusively of responders, and was characterised by a signature with a high abundance of Clostridiales and a low abundance of Bacteroidales, classified as a type I signature. Importantly, in multivariable-adjusted models, the signature remained significantly associated with response and outperformed other established predictive biomarkers such as mutational load.[2]

This concept is highly relevant, as large-scale efforts are underway to profile gut and other microbiota in human health and disease,[7,85] and profiling of gut, tumour, and other microbiota is being integrated into clinical trial designs for several types of cancer therapy. However, there are important caveats with such an effort, as complexities and questions exist regarding the appropriate timing and intervals for microbiome profiling (which will probably be therapy-dependent), as well as methods used to profile the microbiome (16S sequencing *vs* metagenomics *vs* metatranscriptomics *vs* metabolomic profiling). Standardisation of collection, sequencing, and analysis pipelines will be crucial as we move forward, and it is also likely that microbiota will not be a sufficient standalone biomarker but will be best integrated with other known and novel biomarkers.

Factors affecting the gut microbiota

To optimally understand the influence of the microbiome and how best to modulate microbiota, one must understand the complex array of factors that influence the microbiome itself (figure 2). Large-scale population-based studies have shown that distinct microbial communities are largely shaped by environmental factors, with twin studies showing that heritability accounts for only 2–8% of the variation observed.[7,85,86] Studies outside of cancer therapy have shown that the microbiome varies substantially by ethnicity and geography, which might further complicate or obscure cross-study comparisons.[87,88] Furthermore, underlying factors that drive the microbiome's association with geography and ethnicity, including diet, cultural norms, and genetics, might also influence the microbiome's link to patient health with important implications for clinical applications that are personalised and microbiome-based.[89] Unsurprisingly, given the symbiotic relationship between the gut microbiome and the human host in nutrient digestion, diet is a major determinant of the gut microbiome (table 2).[8,85]

The most well-characterised dietary distinction in microbial landscapes is that between the gut microbiome of traditional agrarian communities with a plant-based diet and that of omnivores that are found in industrialised nations.[90] Diets largely composed of fibre-rich plant foods (vegetables, fruits, whole grains) and low in processed foods (refined grains, added sugars, trans fats), with protein primarily derived from fish and plant sources (pulses and legumes) are linked to reduced risk of cardiovascular disease and cancer and decreased overall mortality.[90,91] That the microbiome is a key mechanism in the pathology of diet and these diseases is now well accepted.[32,33,90] Microbial-derived compounds or metabolic products are capable of exerting a number of tumour suppressive and immune-modulating effects (eg, induction of T-regulatory cells, maintenance of epithelial barrier and gut integrity, histone deacetylase inhibition, and suppressing inflammation).[92]

However, it is important to note that commensal bacteria alone are neither good nor bad, per se; rather, our diets play a key role in dictating whether the microbiota present produce beneficial or deleterious metabolites.[37] For example, certain *Clostridium* species produce secondary bile acids in response to dietary fat, or butyrate in response to fibre.[37,93] Further, although the composition of the microbiome can be highly variable between individuals with different diets, there are a number of conserved or redundant functions designed to support the survival of the host and the microbiome (table 2).

Medications are another key determinant of the gut microbiome, particularly antibiotics, which alter microbiome composition and decrease diversity.[1,62,65,94] Importantly, antibiotic use has been associated in some (but not all) studies with reduced response to checkpoint inhibition and an unfavourable gut microbiome in the cancer population.[1,62,63,65] Although this observational association could certainly be confounded by health status necessitating antibiotic use, preclinical models have directly shown that antibiotics can compromise anti-PD-1 efficacy.[1] These data suggest the need for prudence in empiric antibiotic use, particularly broad-spectrum antibiotics with anaerobic activity, in patients with cancer who are receiving immunotherapy. However, infections are also common in patients with cancer, therefore, antibiotics are also critical to reduce morbidity and mortality.

Besides antibiotics, other medications might also impact the gut microbiome, particularly proton-pump inhibitors,[95] but also metformin,[96] anti-depressants,[94] and even hormones.[94] Ultimately, continued investigations of the influences of the gut microbiota on neoplastic and immune cells within prospective studies of exogenous and endogenous factors (eg, diet, lifestyle, and obesity) and cancer outcomes is likely to improve mechanistic understanding and therapeutic precision.[97,98]

	Microbial metabolites	Examples of microbes involved
Fibre-rich plant foods (legumes, cruciferous vegetables, whole fruits, and whole grains)	Short-chain fatty acids (eg, butyrate, propionate, acetate)	*Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia intestinalis, Rumminococcus* spp., *Clostridium* spp.
Polyphenols from soy, cruciferous vegetables, berries, coffee, wine, chocolate, and nuts	Phenolic compounds (eg, urolithin)	*Akkermansia muciniphila, Bacteroides thetaiotaomicron, Bacteroides vulgatus, Bifidobacterium* spp.
Red meat, processed meats, and animal products high in saturated fat and cholesterol	N-nitroso compounds, secondary bile acids (eg, deoxycholic acid) and trimethylamine	*Peptostreptococcaceae, Clostridium* spp., *Fusobacterium nucleatum, Pseudomonas* spp., *Desulfovibrio desulfuricans*

Table 2: **Dietary components and their relationships with microbial metabolism**

Modulating the gut microbiome to improve therapeutic response in cancer

Faecal microbiota transplant

Although faecal microbiota transplant is just beginning to be investigated in the context of cancer, this therapy has been extensively studied in dysbiotic gastrointestinal diseases, specifically *Clostridium difficile* infection[24] and inflammatory bowel disease.[25] Knowledge gained from faecal microbiota transplant in these diseases might inform trial design in the treatment of cancer.

With faecal microbiota transplant, an entire enteral microbial ecosystem is transplanted from the donor or donors, offering multiple potential advantages over delivery of single beneficial bacteria. First, engraftment of introduced bacteria might be more robust in the setting of whole community transplantation with lower competitive exclusion by the recipient microbiome. From an ecological perspective, substantial functional redundancy and interdependence of microorganisms in the overall ecosystem exists, which works cooperatively. While there is considerable overlap between the taxa that have been identified as beneficial, there are also differences at a taxonomic level, which might be reduced at the functional level (table 2). Transplanting an entire ecosystem allows for the transplant of both the putative beneficial bacteria (which could just be biomarkers of the overall health of the ecosystem) and the entire supporting ecosystem and overall diversity (table 3).

Notably, in the profoundly dysbiotic state of recurrent *Clostridium difficile* infections, treatment with a single infusion of healthy donor faecal microbiota transplant results in both clinical benefit and durable microbiota engraftment.[24,99] However, in other diseases in which the native microbiota has not been repeatedly disrupted by antibiotics and dominated by a single pathogenic strain, durable engraftment with single faecal microbiota transplant has proven more challenging.[25,26] Although the use of antibiotic ablation might increase engraftment by reducing competition, there could also be indirect effects of the antibiotics that could compromise therapeutic response (depending on the method used to reconstitute the microbiome).

Ultimately, there are several parameters that must be carefully considered in designing trials of faecal

	Advantages	Disadvantages	Considerations
Faecal microbiota transplant	Transplantation of entire ecosystem; direct	Scalability (difficult to access and expensive); procedural risks; potential to transfer other diseases	Donor selection (complete responder to faecal microbiota transplant *vs* healthy donor); delivery mechanism; need for conditioning regimen; how to sustain; banking for potential future autologous transplant
Probiotics and bacterial consortia	Easy to use; affordable; accessible	Variable engraftment in setting of competing commensals; potential for lowering overall microbiome diversity; varying bioavailability; insufficient regulations on quality control	Use of spores *vs* live bacteria; which bacteria to include; personalisation; need for conditioning regimen
Prebiotics (eg, fibre supplements)	Easy to use; affordable; accessible	Whole food might be more important than isolated nutrient supplements (regulated as food rather than drugs)	Single fibre *vs* mixture; predictability of response given resident bacterial community
Diet	Holistic change that might have other health benefits	Low compliance; difficult to sustain; varied effects	Whether to target specific nutrients *vs* overall pattern; dose needed for target modulation; duration needed; predictability of modulation given host variation in microbiome and metabolism

Table 3: **Methods of microbiome modulation**

microbiota transplant interventions to improve responses to cancer therapy (including immune checkpoint blockade); however, efforts in cancer have not been underway for long and optimal strategies are unclear (figure 3). Donor selection for these studies will be crucial and might be more difficult than for conventional indications for faecal microbiota transplant, characterised by a profound dysbiosis (such as *C difficile*). Several trials in which faecal microbiota transplant is being tested in patients on immune checkpoint blockade are currently underway (eg, NCT03353402 and NCT03341143), which all use complete responder donor faecal microbiota transplant (table 1). Screening of patients by profiling of the microbiome has also been proposed, although there is not a complete understanding of what constitutes a favourable versus an unfavourable microbiome; therefore, this concept needs to be further explored. Clearly, a fundamental and critical component of early trials of faecal microbiota transplant for cancer involves intense biomarker assessment (with longitudinal sampling of faecal samples, blood, and ideally tumours) to assess the effect of the intervention on engraftment, overall immunity, and anti-tumour immunity.

Probiotics

Probiotics are defined as live microorganisms that putatively confer a health benefit to the host to whom they are administered. Probiotics can be either in the form of a supplement or fermented food (eg, yogurt, kefir, sauerkraut, kombucha, miso, or kimchi). The advantages of probiotic supplements are ease of use and accessibility; however, there are substantial issues and variation with standardisation, quality control, bioavailability, and target modulation (table 3).[100]

The earliest commercial probiotic supplements were single strains of easily cultured bacteria derived from food sources, such as the lactic acid bacilli *Bifidobacterium* and *Lactobacillus*. These probiotics have been well studied in the context of many gastrointestinal diseases, with varying levels of evidence for clinical efficacy in the context of specific diseases.[101–103] However, probiotics differ in their ability to survive gastric acids and colonise the intestinal tract, varying on species, dose, and preparation, as well as the native microbiome of the host into which they are introduced.[31,100,104,105]

The remarkable efficacy of orally administered single-strain probiotics in mice on improving anti-tumour immune response supports clinical investigation in cancer populations.[1,21] However, probiotic colonisation in a human with a diverse native gut ecosystem is considerably more challenging.[36] Although antibiotic ablation might be used as a so-called gut conditioning regimen to increase colonisation, a study has shown that probiotics might actually decrease reconstitution of a diverse microbial ecosystem after antibiotics,[106] and thus these approaches need to be carefully tested in clinical trials.

Off-trial use of probiotics in the treatment setting with immunotherapy should be discouraged, because there are limitations of our knowledge on how these microorganisms might affect immunity and therapeutic responses. Additionally, studies have shown that quality can vary greatly, as these supplements are largely unregulated in the EU and the USA.[107] This variability can affect the efficacy and safety of these products and is especially concerning in a susceptible patient population, such as those with cancer (table 3).[107]

So-called designer probiotics or synthetic stool have sought to combine the benefits of faecal microbiota transplant in delivering a diverse ecosystem with the advantage of being able to manufacture such a product to scale with minimal lot-to-lot variation (ie, consistent composition). Early results with these products in recurrent *C difficile* infections show promising stable engraftment of a diverse microbial community.[108] Technological advances have also supported the ongoing development of commensal bacteria that is conventionally unculturable into probiotics, such as the obligate

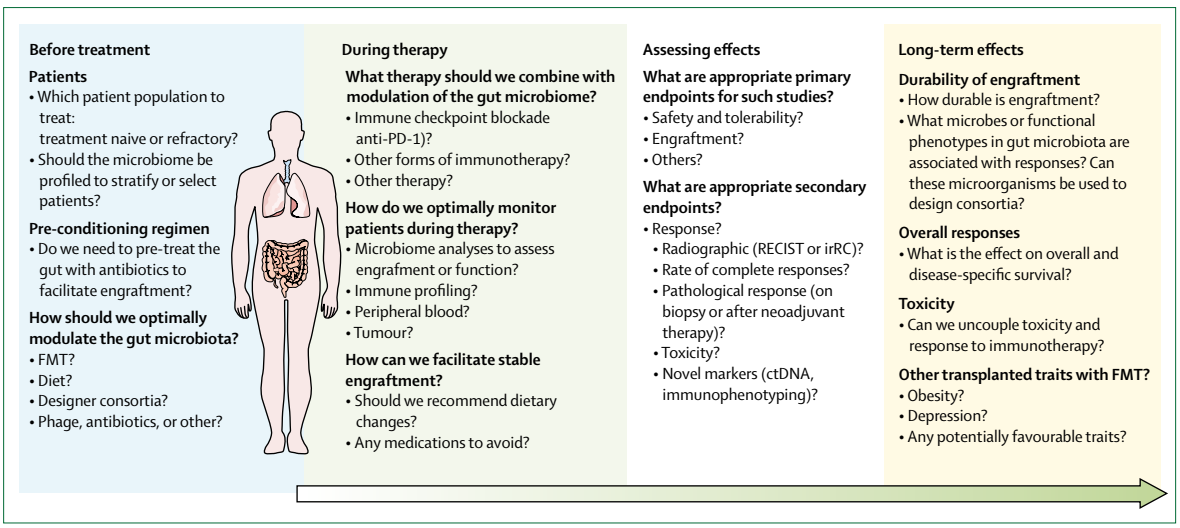

Figure 3: **Questions to consider in clinical trial design for microbiome modulation in cancer**
FMT=faecal microbiota transplant. ctDNA=circulating tumour DNA. RECIST=Response Evaluation Criteria in Solid Tumors. irRC= immune-related response criteria. PD-1=programmed death receptor-1.

anaerobe bacteria, *Faecalibacterium prausnitzii,* which ferments fibre, which has been associated with favourable immunotherapy response.[2,3,109] Finally, testing of postbiotics (defined as metabolic by-products from microorganisms with beneficial biological activities such as butyrate) have been proposed; however, consistent data to support this approach are scarce.

Diet and prebiotics

The gut microbiome's growing role in personalised cancer medicine is shaping new priorities for diet research in patients with cancer. There is no specific or evidence-based dietary guidelines to share with patients following their diagnosis, despite desperate inquiries and the widely-held conviction that diet is an important component of health. This gap is due in part to insufficient dietary data collection in trials and clinical cohorts and a scarcity of prospective interventional dietary studies showing change in cancer response and survival outcomes.[91]

Although specific bacterial taxa differ in responders to immune checkpoint blockade in many studies (with *Faecalibacterium prausnitzii, Akkermansia muciniphila, Bifidobacterium longum,* and members of the *Clostridium* genera identified across the cohorts), different bacterial taxa shared some commonalities in their links to upstream dietary substrates and to downstream pathways (table 2).[110]

Many foods (eg, processed meats) or components of food (eg, dietary fibre) have been identified that increase or decrease the risk of developing cancer.[91] However, it appears increasingly unlikely that specific foods, nutrients, or other bioactive food-derived compounds are themselves important singular factors in promoting or inhibiting cancer; rather, different patterns of diet combine to create a metabolic and inflammatory state that is more or less conducive to tumour progression. Diets that score well across a range of parameters and international recommendations (eg, Mediterranean diet score and the Healthy Eating Index) are associated with substantially reduced risk of developing and dying from cancer[91] and have also been linked to lower inflammation and enhanced immune function via cytotoxic and T-helper cells.[111] However, there remains considerable interest whether specific dietary components might favourably alter the microbiome (table 2). Randomised controlled trials testing addition of specific foods or nutrients—eg, substitution of whole grains for refined grains—have shown modest changes in the microbiota and immune function.[112,113] However, several short-term dietary studies with gut microbiome endpoints have shown that dramatic shifts in diet (eg, putting a vegetarian on a meat-based diet or a >30% energy restriction) can have equally dramatic effects on the microbiome, as well as cancer biomarkers such as cellular proliferation.[40,90,114,115] However, these changes in the microbiota are just as rapidly reversible if dietary changes are not sustained. Thus, consistent dietary change would be needed to enrich beneficial bacteria and shape the gut landscape; however, changing long engrained dietary patterns is notoriously difficult. Well designed and controlled studies are needed to improve our understanding of the importance of diet-induced changes on the composition and function of the gut microbiome and the effect of these changes on response to cancer therapies (table 3).

In addition to dietary modulation, administration of prebiotics (nutrients such as resistant fibres) that enrich for putatively beneficial gut microbiota are also being studied in the context of the microbiome and cancer

Search strategy and selection criteria

References for this Review were identified through searches of PubMed using the search terms "microbiota", "microbiome", "cancer", "immunotherapy", "chemotherapy", "radiotherapy", "stem-cell transplant", "fecal microbiota transplantation (FMT)", "probiotics", "prebiotics", and "antibiotics". No date limits were applied. Articles were also identified through searches of the authors' own files. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review. Additionally, ClinicalTrials.gov was searched for trials of microbiome modulation in cancer using the terms "microbiota", "microbiome", "cancer", "fecal microbiota transplantation (FMT)", "diet", "probiotics", and "prebiotics". We included all interventional trials with microbiome listed as either a primary or secondary outcome. Observational studies were excluded.

as are synbiotics (NCT01929122, NCT01549782, NCT03420443, NCT03072641, and NCT01479907; table 1). However, just as with single-species probiotics, targeted prebiotics might not enrich the overall diversity of the microbiota or the other clans of bacteria that might have complementary metabolic functions (table 3).

Conclusion

There is compelling evidence that the microbiota affects immunity and therapeutic response in cancer, and that manipulation of microbiota can augment response to immunotherapy in preclinical models. Experience in other diseases, including *C difficile* colitis, has shown that the microbiome can indeed be successfully modulated to alter pathophysiology and benefit patients, and clinical trials targeting this approach for patients with cancer are in development or underway. However, we must recognise that there are unique considerations that vary by disease and patient population. The underlying degree of dysbiosis might influence amenability to modulation. Thus, optimal approaches are unknown and complexities exist regarding ideal strategies to be used (figure 3).

Certainly, faecal microbiota transplant has shown efficacy in some non-cancer indications where profound dysbiosis exists; however, this approach might require more nuance in patients with cancer with regard to the selection of the most appropriate target population as well as potential donors. Administration of bacterial consortia might seem more appealing (either with available off-the-shelf probiotics or with so-called designer consortia); however, there are considerable questions regarding the efficacy of this approach and the optimal composition of these formulations. Additionally, studies suggest highly variable colonisation and functional effect of empiric probiotics.[36] Thus, use of off-the-shelf probiotics outside of a clinical trial should be discouraged. Finally, although dietary changes might offer off-target health benefits, whether diet is sufficient to modulate the target in the setting of cancer therapy also needs to be shown in a trial setting.

Although important insights have been gained regarding the role of the gut microbiota in response to cancer therapy, there is still a tremendous amount to learn. This gap includes fully identifying the components of the human gut microbiota, including all bacterial as well as viral, archaea, protozoal, and fungal components, and how these components interact and influence one another and the overall immune response. Moreover, differences in processing and analysis can limit cross-study comparison. For example, although multiple groups have identified specific bacteria that are associated with response to immunotherapy in patients and mice, there is little consistency across these studies, and it is unclear to what extent this inconsistency is driven by differences in sequencing methodologies (16S with different variable regions used or whole-genomic sequencing) or rather differences in host interaction with environment, diet, tumour type, etc, since the patient populations in these studies varied substantially according to these factors. Sampling the gut microbiota of a broader range of patients, disease types, and stages in a systematic, and perhaps more crucially, standardised, way will facilitate consensus and further our understanding. Importantly, standardisation of sequencing techniques must happen at every level of analysis, from sample collection to DNA isolation to sequencing technique used to data analysis pipeline and library choice. Ultimately, the inconsistency in specific favourable bacterial species identified in different cohorts might represent an example of "function over phylogeny"[116] meaning that the individual components of the microbiota do not matter as much as their combined (and often redundant) functions. Hopefully, examining the gut transcriptome, proteome, and metabolome will assist in this understanding.

Culturing individual bacteria and further development of culturomics will enable us to study the bacteria themselves rather than their genomes only and approach mechanisms in a way that computational methods simply do not allow. Additionally, developing more and better mouse models will be crucial to help elucidate mechanism. Although important thought-changing studies have been garnered using germ-free mice, this model system is far from perfect. Indeed, germ-free mice have profound defects in local (gut) and systemic immunity. Furthermore, similar germ-free systems must be developed to study the effects of viral and fungal components.

Ultimately, it is only through standardisation of approaches, extensive corollary analyses of biospecimens obtained from trial participants, and the global integration of available data that we will be able to derive actionable strategies targeting the microbiome in cancer therapy.

Contributors
JLM and JAW contributed to the conception of the review. All authors contributed to the writing of the manuscript and review and revision of the manuscript. JLM, BH, and JAW contributed to the creation of the figures.

Declaration of interests
JAW is an inventor on a US patent application (PCT/US17/53.717) submitted by the University of Texas MD Anderson Cancer Center that covers methods to enhance immune checkpoint blockade responses by modulating the microbiome. JAW is also a paid speaker for Imedex, Dava Oncology, Omniprex, Illumina, Gilead, MedImmune, and Bristol-Myers Squibb, a consultant and advisory board member for Roche-Genentech, Novartis, Astra-Zeneca, GlaxoSmithKline, Bristol-Myers Squibb, Merck, and Microbiome DX, reports clinical trial support from GlaxoSmithKline, Roche/Genentech, Bristol-Myers Squibb, and Novartis, and is a clinical and scientific advisor at Microbiome DX and a consultant at Biothera Pharma, Merck Sharp and Dohme. All other authors declared no conflicts of interest.

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