Gut microbiome in modulating immune checkpoint inhibitors

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Summary

Gut microbiome has been increasingly recognized for its influence on a diverse array of human diseases including cancer, and may also influence the outcome of cancer therapies. A prime example is seen in immunotherapy, for which gut microbes determine the therapeutic responses associated with immune checkpoint inhibitors (ICIs) in preclinical models and patient cohorts. This evidence hints that inter-individual variations in the gut microbiota may account for the significant heterogeneity in immunotherapeutic responses to ICIs. Understanding the functional role of gut microbiome in regulating not only mucosal but also systemic immunity and cancer is critical to move forward in this era of precision medicine. What's more, microbiota can be modified via several different strategies that are essential for the efforts in expanding immunotherapy efficacy. This review summarizes latest knowledge about the interactions between microbiome, host immunity and cancer, and strategies to modulate the microbiome with implications to be translated into clinic.

Keywords: Gut microbiome; Host immunity; Checkpoint inhibitor; Fecal microbiota transplantation; Probiotics; Prebiotics

Introduction

In the past two decades, immune checkpoint inhibitors (ICIs) have shown to dramatically improve survival of patients with multiple types of cancers, especially in locally advanced and metastatic settings.[1,2] However, the efficacy of ICIs is largely compromised by the immune-related adverse effects on the host tissues. In recent years, mounting evidence has suggested that gut microbiome, a collection of microbes and its metabolites, profoundly influences immune system, thus affecting the efficacy of ICIs.[3] Investigation of the reason why this occurs requires insight into the complex intrinsic link among gut microbiome, cancer and anti-tumor immunity of the host. Herein, we will discuss how these factors form strong associations and how they influence each other as well as the therapeutic response of ICIs. We will also assess the up-to-date evidence that successful modulation of the gut microbiota can improve the outcomes of patient with ICIs, and highlight promising approaches that might be translated to novel avenues for cancer immunotherapy.

Gut microbiome and host immunity

Through an enduring mutualistic partnership, the crosstalk between the gut microbiome and the host immune system has evolved into a multifold network, which plays an important role in maintaining the delicate balance of tolerance of commensal microbiota and food antigens and defense against various potentially pathogenic bacteria and external perturbations such as medication. Nonetheless, perturbations of gut microbiota and impairment of the integrity of the mucosal barrier can cause dysbiosis, resulting in immune-related
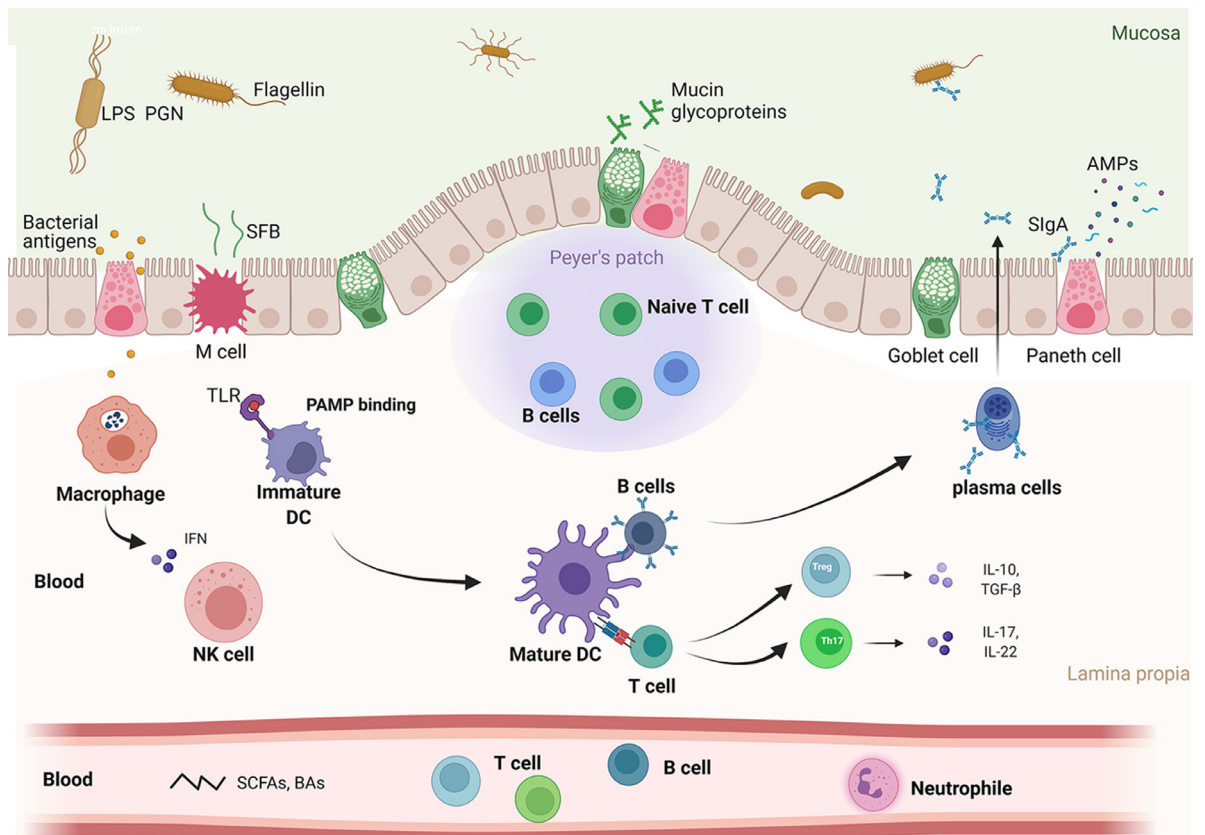
Figure 1. Gut microbiome and host immunity. The intestinal microbiota can profoundly affect the activation of both innate and adaptive immunity at the local level in the mucosa, lamina propria, Peyer's patches, and mesenteric lymph nodes, leading to systemic immune dysregulation. The gut microbiota helps to create a thick mucus protective layer composed by mucins glycoproteins released from Goblet cells. Microbial antigens like Flagellin, PGN or LPS can induce production of AMPs secreted from Paneth cells and epithelial cells. Signals from the gut microbiota can also be transferred by DCs and macrophages and contribute to the development of inflammatory T-cell subsets, including Tregs, Th17 cells, etc. B cells are also activated directly by microbial antigens and/or with the help of mature DCs, differentiating into plasma cells and producing protective sIgA. Besides, activation of naive T cells and B cells also occurs in Peyer's patches when their cell receptors encounter appropriate APCs, which triggered by PRRs through PAMP detection of microorganisms. SFB are potent inducers of Th17 cells, whereas important microbial metabolites such as SCFAs stimulate Treg-cell differentiation. Th17 cells can produce IL-17 and IL-22, promote inflammation at the local site and function in recruiting neutrophils from the blood. In the contrast, Tregs secret IL-10 and TGF-β, which can inhibit the activity of multiple immune cells and create an anti-inflammatory cytokine milieu. Translocation of some bacterial metabolites, such as SCFAs and BAs from the gut lumen to the lymph nodes and blood, can further shape the systemic immunity. Abbreviations: PGN, peptidoglycan; LPS, lipopolysaccharides; AMPs, antimicrobial peptides; DCs, dendritic cells; sIgA, secretory IgA; APCs, antigen presenting cells; PRRs, pattern recognition receptors; PAMPs, pathogen-associated molecular patterns; SFB, Segmented filamentous bacteria; SCFAs, short chain fatty acids; BAs, bile acids. Created with BioRender.com.

disorders and increased risk of cancer. Growing evidence of numerous molecular mechanisms reveals that gut microbes might influence host immunity in health and cancer, both within and outside the gastrointestinal tract. Overall, there's a strong necessity for us to discuss the recent advances in the role of gut microbiota in shaping the host immune system (Figure 1).

Gut microbiome and innate immunity
The innate immunity provides a primary host response to microbial invasion in which microbial-derived agents are recognized by germline-encoded pattern recognition receptors (PRRs) expressed on epithelial cell as well as innate immune cells within the gut. These PPRs could detect pathogen-associated molecular patterns (PAMPs) of microorganisms, including lipopolysaccharide, flagellin, peptidoglycan, formyl peptides and unique nucleic acid structures.[4] The intracellular signaling cascades triggered by PRRs can thus induce the transcriptional expression of inflammatory mediators to prevent systemic dissemination of pathogens.[5] The inflammatory response is orchestrated by proinflammatory cytokines such as type I interferons (IFNs), chemokines and proteins involved in the

modulation of PRR signaling, which can also promote the differentiation of T cells and B cells to establish antigen-specific adaptive immunity.[5] For example, a pilot study investigating the fecal microbiota of HIV-1-infected patients found that *Prevotella* levels were associated with the IFN-I pathway and T cell responses, suggesting that *Prevotella* may become a probiotic as a therapeutic strategy for HIV.[6]

Gut microbiome and adaptive immunity

In the largest proportion of the human immune system, gut-associated lymphoid tissues (GALT), which include the lamina propria and Peyer's patches (PP), triggering of PRRs expressed both on cell surface and inside the cell are capable of inducing the functional maturation of (dendritic cells) DCs and priming of naïve T cells and B cells, and thus coupling innate and adaptive immunity.[7] Luminal antigens are sampled directly by DCs through extension of dendrites (membrane extensions) between epithelial cells, or indirectly by endocytosis of specialized antigen-sampling cells (called M cells) via transcytosis relatively intact to DCs and macrophages. After being primed, naive T and B cells are differentiated into T regulatory (Treg) cells or effector T cells and IgA-producing plasma cells which migrate from the efferent lymph vessels of the GALT to the mesenteric lymph nodes (mLN), and finally to peripheral blood via the thoracic duct and therefore involving in host systemic immunity.[8] Pinacho et al. reported that the abundance of the genus Prevotella may influence the intestinal mucosal T cell response, highlighting the crosstalk between the gut microbiota and host immune system.[6]

The lamina propria and Peyer's patches contains a large number of IL-17$^+$CD4$^+$ T (Th17) cells and Foxp3$^+$ Treg cells, which represent a class of potent immunomodulatory effector cells. In particular, Th17 cells are a specific lineage of CD4$^+$ TH cells that are essential for host defense and play a key role in the development of autoimmune disease by producing the pro-inflammatory cytokines interleukin-17A (IL-17A), IL-17F and IL-22.[9] The study in germ-free models has demonstrated that Th17 cells were induced upon colonization of commensal bacteria, especially for segmented filamentous bacteria (SFB).[10,11] Further, Treg cells and Th17 cells can promote class switch of B cells and production of secreted IgA (SIgA), therefore contributing to compartmentalization of commensal microbiota and their homeostasis within local habitats.[12−14] The coating of commensal bacteria and their soluble antigens by SIgA inhibits their binding to the intestine epithelium and penetration into the lamina propria.[15] Thus, the production of SIgA is crucial for host-commensal mutualism maintenance and function of intestinal mucosal barrier in protection against pathogen invasion.[16−18]

Commensal bacteria can enhance intestinal epithelial cell barrier function through production of a diverse array of metabolites. For instance, short-chain fatty acids (SCFAs), namely acetate, propionate and butyrate,

function as energetic substrates for epithelial cells. For example, acetate produced by *Bifidobacterium spp*, could inhibit the translocation of Shiga toxin produced by *E. coli* O157:H7,[19] while bacterially produced butyrate has been reported to regulate energy metabolism in intestinal epithelial cells (IECs), by serving as primary energy source for colonocytes.[20]

Gut microbiome's role in (tumor microenvironment) TME

Dysbiosis of microbial community usually induce the disruption of the intestinal barrier facilitating subsequent leakage of microbes and metabolites, which leads to chronic inflammatory state, lipid metabolism disorders, deregulation of growth of cells and impairment of the ability of myeloid cells in clearing mutant, senescent and malfunctioning cells, thereby promoting tumor outgrowth.

Reconstitution of germ-free mice with patient-derived microbiota has revealed a mechanistic link between microbiota composition and anti-tumor immunity.[21] Gut commensals-derived MAMPs or PAMPs can traverse the mucosal barrier, enter the circulation and reach to distant sites such as lymph nodes and tumor where a strong immune response is triggered.[22] Recent studies suggested the concept of antigen cross-reactivity as a factor of augmenting anti-tumor immunity.[21,23] Under this proposed model, cross-reactive T cells primed against bacterial antigens might activate anti-tumor response either by providing help (CD4$^+$ T cells) or through direct killing (CD8$^+$ T cells). A pancreatic cancer-related study demonstrated that intra-tumoral and circulating T cells are responsive to both neoantigens and predicted cross-reactivity with microbial epitopes.[23] Another candidate mechanism by which gut bacteria modulate anti-tumor immunity is through local induction of immunomodulatory cytokines released by host cells (such as gut epithelium or immune cells) that disseminate systemically. These cytokines, including TNFα, TGF-β, IL-12 and IL-10, may shift the threshold of immune subsets activation within the tumor microenvironment, thereby resulting in augmented adaptive immune responses. Additionally, the gut microbiota can also influence anti-tumor immunity by releasing various metabolites that can enter the host circulation. A prominent example is the effect of SCFAs which can affect macrophage and DC function,[24,25] activation of anti-inflammatory T-regulatory (Treg) cells or pro-inflammatory T helper (TH1) and TH17 cells,[26] as well as IgA secretion by plasma cells[27] in TME and tumor-draining lymph node (TdLN).[28]

Modulating gut microbiota to induce cancer immunotherapy

Although the immunotherapy with checkpoint inhibitors has seen unprecedented clinical efficacy, a large
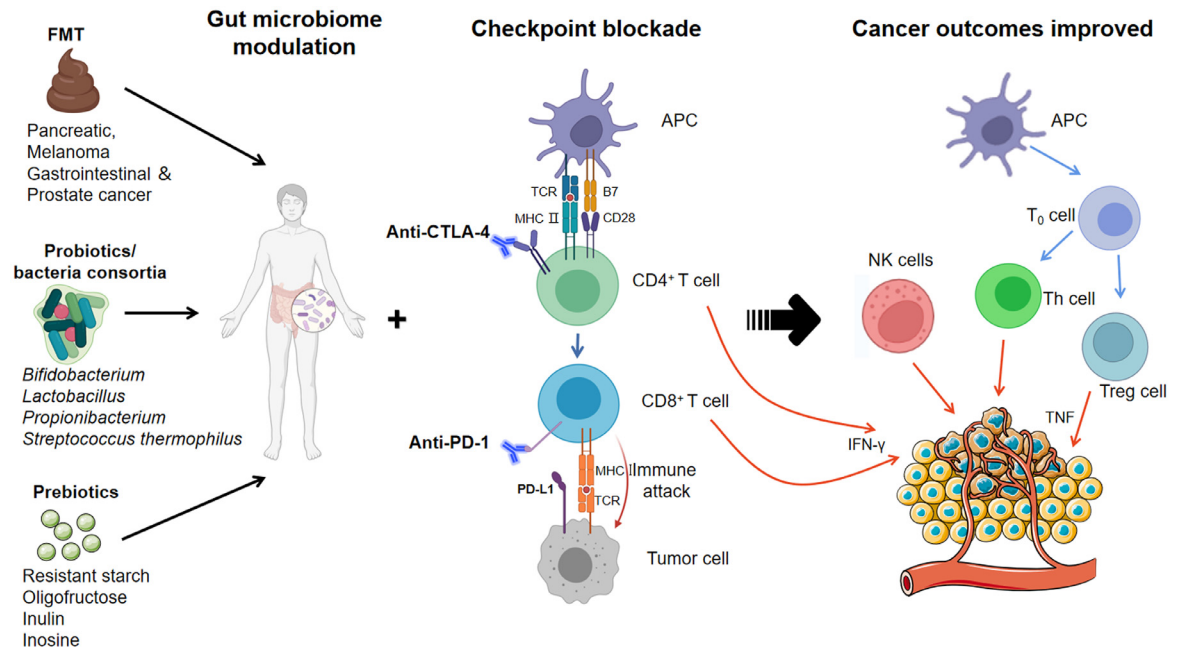
Figure 2. Gut microbiota modulation improves the efficiency of cancer immunotherapy. Different strategies can be used to regulate the gut microbiome and can be used as interventional measures to improve the efficiency of cancer immunotherapy. i.e., FMT in combination with checkpoint inhibitors are able to reprogramme the tumor microenvironment and activate host immunity with favorable changes in immune cell infiltrates in patients with prostate cancer, melanoma, gastrointestinal and prostate cancer. Probiotics and/or bacteria consortia containing live bacteria such as *Bifidobacteria, Lactobacillus, Propionibacterium* and *Streptococcus thermophilus* combined with monoclonal antibodies (anti-PD-1 and anti-CTLA-4 antibodies) can significantly improve the outcomes of cancer patients receiving immunotherapy and probiotics vs immunotherapy alone, reflected in the significant alterations in the level of inflammatory cytokines including IFN-γ and TNF, etc. Additionally, prebiotics (RS, oligofructose and inulin, etc) are capable of facilitating the growth of selective healthier microorganisms and their metabolites (eg. SCFAs, inosine) and enhance tumor cell killing efficacy through promoting both effector and regulatory T lymphocyte subsets, depending on the cytokine and immunological milieu. Abbreviations: FMT, fecal microbiota transplantation; PD-1, programme cell death-1; CTLA-4, cytotoxic T-lymphocyte associated protein-4; IFN-γ, interferon-γ; TNF, tumor necrosis factor; RS, resistant starch; SCFAs, short chain fatty acids.

portion of responders would develop acquired resistance after initial response. As described above, gut microbiome profoundly influences host anti-tumor immunity in a variety of ways, thereby impacting the clinical responses and outcomes of the patients receiving cancer immunotherapy. Largely based on that the specific interventions with the potential to modulate the gut microbiota may develop new treatments as an important adjunct to current anti-cancer therapeutics, efforts are currently underway with several ongoing and planned clinical trials to improve the therapeutic responses and/or abrogate treatment-associated toxicity via manipulating gut microbiota directly in cancer patients (Figure 2, Table 1).

Fecal microbiome transplantation
FMT represents the most direct means to manipulate the microbiota, stool from a given donor is transferred to a recipient through oral administration of lyophilized or frozen pills or through direct delivery by colonoscopy or gastroscopy. To date, FMTs are explored as a therapeutic option for a growing list of indications with roughly 300 registered clinical trials (clinicaltrials.gov, accessed Aug 2021). Within the past decade, FMTs have been shown to be incredibly highly effective in the treatment of resistant and recurrent *Clostridium difficile* infection, thus improving patients' status and resolving clinical symptoms.[29−32] Recent studies have revealed that germ-free mice receiving FMT from PD-1 blockade responsive patients could restore enhanced anti-tumor immunity and be responsive to anti-PD-1 therapy. On the contrary, germ-free models treated by stool material from non-responsive patients could not successfully form the response to PD-1 blockade.[21,33,34] Furthermore, a preclinical study was conducted in which germ-free mice was transferred with fecal microbiota specimens from long-term survivors vs short-term survivors with pancreatic adenocarcinoma, and the result revealed that FMT could reconstitute not just the gut microbiome in the mice but also the microbiome, growth, and immune cells infiltration of the tumor.[35]

NCT number	Patients	n	Intervention	Outcome measures	Status
NCT03353402	Melanoma patients who failed immunotherapy	40	FMT from immunotherapy responding patients	Primary: Incidence of FMT-related AEs; Proper implant engraftment Secondary: Changes in immune cell population and activities	Recruiting (Israel)
NCT04758507	Renal cell carcinoma patients received ICIs	50	FMT(from donors who are responding to ICIs))	Primary: CRR Secondary: ORR; OS; Rate of AEs Rate of increase in α-diversity and β-diversity of their gut microbiota	Recruiting (Italy)
NCT04130763	Gastrointestinal Cancer patients who failed anti-PD-1 treatment	10	FMT capsule combined with anti-PD-1 therapy	Primary: ORR; Rate of abnormal vital signs and laboratory test results; Rate of AEs Secondary: Change in T-cells Composition, CD8+ T-cell receptor diversity and CD56 + NK cells,etc; Function of T-cells; Association of anti-PD-1 response with gut microbiota	Recruiting (China)
NCT04924374	Patients with advanced lung cancer who received immunotherapy	20	FMT from healthy donors or long-term survivors to advanced lung cancer	Primary: Measure of safety Secondary: Measure of efficacy (CRR and ORR)	Recruiting (Spain)
NCT04729322	Anti-PD-1 non-responders in colorectal cancer patients	15	FMT from donors who responded to PD-1 antibody	Primary: ORR	Recruiting (USA)
NCT05032014	Liver cancer patients who received anti-PD-1 treatment	46	Probiotic-M9 (Lactobacillus rhamnosus)	Primary: ORR Secondary: PFS;OS	Recruiting (China)
NCT05094167	NSCLC patients who received PD-1 inhibitor and platinum	46	Probiotic-V9(Lactobacillus Bifidobacterium)	Primary: ORR Secondary: PFS;OS	Recruiting (China)
NCT04909034	NSCLC patients who received Pembrolizumab	30	Fermented soybean extract MicrSoy-20(MS-20)	Primary: The incidence of AEs Secondary: ORR; PFS; DCR; DOR	Recruiting (Taiwan)
NCT04552418	Patients with solid tumor who received dual ICIs	12	Potato starch (Resistant starch)	Primary: Compliance;AEs of ICI therapy; unanticipated AEs Secondary: Incidence and severity of ICI therapy -induced diarrhea/colitis; Change in microbiome composition and metabolite	Recruiting (USA)

Table 1 (Continued)

NCT number	Patients	n	Intervention	Outcome measures	Status
NCT05083416	Head and neck cancer patients who received immunotherapy	52	Prolonged nightly fasting; Regular eating pattern	Primary: Rates of prolonged nightly fasting (PNF) compliance; Change in gut micro-biome and microbial metabolites	Recruiting (USA)

Table 1: **Clinical trials on gut microbiome modulaton in cancer immunotherapy.**
Abbreviations: ORR, objective response rate; CRR, complete response rate; DCR, disease control rate; DOR, duration of response; OS, overall survival; PFS, progression-free survival; NSCLC, Non-Small Cell Lung Cancer; FMT, fecal microbiota transplant; PBMC, peripheral blood mononuclear cell.

These observations provide a strong paradigm for studying how a favorable gut microbiome can be restored through clinical interventions.

Clinical trials on the potential of FMT to improve immunotherapy efficacy in patients with tumor recurrence or therapeutic resistance are underway in several cancer types including melanoma, gastrointestinal and prostate cancer. The first move in the general protocol for these clinical studies is to identify the fecal donor candidates with fecal samples and a microbial composition that meet the defined quality control and test standards, as well as obtaining an complete response to their immunotherapy. A phase I clinical trial was performed to assess the reinduction of anti—PD-1 immunotherapy in 10 patients with anti—PD-1—refractory metastatic melanoma. The researchers reported that clinical responses were observed in three patients, including two partial responses and one complete response. Notably, they further evaluated the gut and tumor biopsies and found that FMT was associated with favorable changes in immune cell infiltrates and gene expression profiles in both the gut lamina propria and the tumor microenvironment (NCT03353402).[36] Another melanoma trial out of University of Pittsburg evaluating FMT together with pembrolizumab in melanoma patients primarily resistant to PD-1 inhibitor therapy showed clinical benefit in 6 of 15 patients. The data further revealed that FMT shifted the microbiota of recipients toward a donor-type taxonomic composition involving the activation of the host immunity and alterations in the host metabolism (NCT03341143).[37] Overall, these findings suggest that FMT in combination with PD-1 blockade is able to change the gut microbiome and reprogramme the tumor microenvironment to mitigate the resistance against anti-PD-1 therapy in patients with advanced and/or metastatic melanoma.

Despite the data from clinical trials of FMT treatment on patients under cancer immunotherapy seems provocative, the safety of FMT remains a concern for investigators. Since FMT has just been used as a therapeutic approach recently, there is a lack of long-time safety trials and a series of adverse events were reported. For instance, FMT administration of patient with ulcerative colitis or *Clostridium difficile* infection showed minor adverse effects including abdominal cramps, bloating, and sore throat.[38,39] In addition to abdominal discomfort, more serious adverse events like bacterial infections were also noted, possibly caused by the transfer of pathogenic bacteria, drug-resistant bacteria, parasites and bacteriophage from fecal donor. In two separate clinical trials, two patients developed extended-spectrum beta-lactamase -producing *E. coli* bacteremia after they had undergone FMT, and one of them died from severe sepsis despite maximum supportive measures.[40] This consequence caused the U.S. Food and Drug Administration issuing a safety bulletin warning on the risk of serious adverse events arising from transmission of pathogenic organisms in FMT (FDA C for BE and. Important Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Risk of Serious Adverse Reactions Due to Transmission of MultiDrug Resistant Organisms. FDA 2019). Since both cases described above were linked to the same stool donor, the FDA's safety bulletin suggests that future inclusion criteria utilizing FMT should strictly avoid the donors harboring the potential pathogenic bacteria. In addition to infection related disorders, some other causes of death after FMT treatment have also been reported. A retrospective study conducted at 16 medical centers nationally and internationally in which two deaths occurred within 12 weeks of FMT, one of them was because of aspiration during sedation for FMT administered via colonoscopy.[41] Although several adverse events were reported during FMT treatment, it is difficult to recognize which one is specifically associated with FMT. Such as vomiting, which occurs frequently in various clinical practices, is regarded as a common host response after FMT administration.[42] Further randomized controlled trials assessing the adverse events more precisely is needed to be conducted in the near future. Moreover, researchers involved in FMT intervention on immunotherapy-refractory patients wonder if they could identify the candidates with immunotherapy resistance are only the result of gut microbiome imbalance, since the primary resistance is also related to tumor-intrinsic oncogenic pathways and certain microenvironmental

factors causing cancer cells immune escape.[43−49] As the progressed understanding of the biochemical and cellular mechanisms of the gut microbiome regulating host anti-tumor immunity, ideal selection of FMT donors is possible in the near future.

Probiotics and bacteria consortia

Probiotics are live microorganisms or a combination of microorganisms that confer a health benefit on hosts when consumed in adequate amounts.[50] Compared to untargeted FMT, probiotics are attempting for specifically modulating the gut microbiome, particularly through adding probiotics to microbial communities. As administration of over-the-counter probiotics or empirically determined clinical probiotic candidates is often considered to be supplements or functional foods, the next-generation probiotics are developed involving single or multi-strain bacterial consortia on the basis of strong scientific rationale and evidence regarding their efficacy.[51] Other than over-the-counter probiotics, next-generation probiotics lies not only in supplementing beneficial functionalities, but also in providing the necessary ecological context to sustain them.

'Several clinical trials have examined the impact of probiotics administration on patients with cancer therapy by assessing the changes in microbiota composition. Research conducted at Sahlgrenska Univ. Hospital from Sweden showed that probiotic intervention to patients with CRC altered the microbial signature within the gut, characterized by enrichment of butyrate-producing bacteria in mucosal and fecal samples (NCT03072641).[52] The probiotic strains of *Bifidobacterium lactis* and *Lactobacillus acidophilus* used in this study are expected to serve as a beneficial component of therapeutic development in CRC.[52] In addition to the microbiota composition assessment, investigators also seek to explore whether there are changes within the tumor microenvironment after adding probiotics to cancer patients as an adjuvant. For instance, patients with operable breast cancer were given 2-4 weeks of probiotics prior to surgery and their alterations in tumor microenvironment were evaluated by counting the average number of cytotoxic T lymphocytes (NCT03358511). Another study showed that probiotics containing six viable microorganisms of *Lactobacillus* and *Bifidobacteria* strains caused significant reduction in the level of pro-inflammatory cytokines, including TNF-α, IL-6, IL-10, IL-12, IL-17A, IL-17C and IL-22 (except for IFN-γ) in CRC patients who received probiotics as compared to pre-treatment level, suggesting that probiotics may modify intestinal microenvironment such as decline in production of pro-inflammatory cytokines (NCT03782428).[53] Moreover, studies are being conducted focusing on differences in outcomes to cancer immunotherapy such as monoclonal antibodies. The first randomized, prospective study of a live bacterial product from *C. butyricum* in combination with dual checkpoint

inhibition (anti-PD-1 and anti-CTLA-4 therapy) treating advanced kidney cancer patients was conducted in City of Hope Medical Center in the U.S, the response rate and median (progression free survival) PFS were both improved among patients receiving dual checkpoint inhibition and probiotic vs immunotherapy alone, revealing that probiotics can help immunotherapy in assisting the body's immune system attacking cancer (NCT03829111) (ASCO 2021). In addition, research institutions and international drug manufacturers from around the world are studying more bacteria consortia in combination with anti-PD-1 treatment for various cancer types. For example, researchers at Jiangxi Provincial Cancer Hospital from China found that regulating the abundance of beneficial bacteria such as *lactic acid bacteria, bifidobacteria and Akkermansia Muciniphila* improved the effect of PD-1 inhibitors on tumors in mice. Therefore, they designed further "paired clinical trials" to study the oral probiotics V9 (*L. Bifidobacterium*) and M9 (*L. rhamnosus*) isolated from healthy female breast milk samples in 2017, combined with PD-1 inhibitors for patients with non-small cell lung and liver cancer. With objective remission rate (ORR) and PFS or OS being calculated, they estimate better prognosis in combination therapy compared to monotherapy (NCT05094167, NCT05032014) (Table 1). In addition to all the aforementioned bacteria favorable to cancer immunotherapy, negative microbes have been reported. In the context of checkpoint inhibitors, *Blautia obeum* is associated with a detrimental immunophenotype and an increased risk of developing ICI-related colitis.[54,55] *Bacteroides* have mixed effects, they are thought to be detrimental, because they can induce the expansion of Treg cells or stimulate the production of anti-inflammatory cytokines, or beneficial.[56] Therefore, the outcome of patients receiving cancer immunotherapy can also be predicted based on these negative markers.

Due to a long use history of traditional probiotics, it remains to be safe in both daily life and clinical administration. But when it comes to next-generation probiotics, they are isolated and identified by a variety of novel tools, such as sequencing techniques and analysis pipelines, which lead to moderate overlaps of the response-related bacterial taxa in the studies conducted so far. Additionally, variations among patient cohorts, such as cancer types, geography, diet and other environmental factors may also contribute to the bacterial taxa overlaps. Therefore, several factors should be incorporated into our consideration in the future analysis, including standardization of microbiome sequencing approaches, analysis of metabolomic profiling and other methods to improve the efficiency of next-generation probiotics.

Prebiotics

Prebiotics, defined as fermentable, non-digestible chemicals or substrates which promote the growth of selective group of microorganisms and thereby a diverse and

'healthy' microbiota, are another means of targeted microbiome modulation.[57] The purpose of prebiotics administration is to confer a selective advantage to beneficial members of the microorganisms compared to the direct use of microbiotics. These prebiotics mainly include carbohydrates which arrive undigested into the large bowel where they are fermented by commensal bacteria. SCFA is produced through this fermentation, leading to a lower pH level in intestine, thus sustaining the growth of beneficial microbes such as *Lactobacillus* and *Bifidobacterium*.[58] As one of the most studied prebiotic, resistant starch (RS) is capable of facilitating the growth of bacteria associated with the butyrate production. Preclinical studies reported that prebiotics appeared to be supportive for several chemotherapies as well as radiotherapy, either in terms of therapeutic efficacy or diminished toxicity. Taper et al. incorporated oligofructose or inulin into the basal diet for liver cancer mice which accepted subtherapeutic dose of multiple common chemotherapeutic agents, including 5-FU, doxorubicin, vincristine, cyclophosphamide, methotrexate, cytarabine, and found a significantly enhanced therapeutic efficacy of six cytotoxic drugs.[59] Moreover, most of studies indicated beneficial effects for prebiotics in terms of improving immune system, by modulating the expression of pro or anti- inflammatory cytokines. For example, butyrate production, facilitated by RS, significantly inhibits the production of proinflammatory cytokines interferon (IFN)-γ and interleukin (IL)-2 in rat represented by lower ratio of IFN-γ to IL-10 in mesenteric lymph nodes (mLNs).[60] The prebiotic combination of inulin and oligofructose described above can also significantly decreased the expression of cecal proinflammatory IL-1β after they were incorporated into the drinking water of rats, which was also supported by other researchers.[61,62] The regulatory function of prebiotics in terms of host immune system indicated a potential therapeutic role in cancer immunotherapy, relevant exploration in clinical studies is processing. For example, a pilot study is underway to assess the gut microbiome modification with administration of resistant starch in patients undergoing treatment for solid cancers with dual ICIs. The frequency of known adverse events (AEs) attributable to ICIs treatment will be compared to historical incidence (NCT04552418). It also has emerged that the purine nucleoside inosine produced by gut microbiota plays a role in the modulation of immunotherapy responses. Recently, Mager et al. identified inosine as a metabolite produced by *Bifidobacterium pseudolongum* that improves the efficacy of anti-tumor responses by immune checkpoint inhibitors in germ-free mice with multiple tumor types, including colorectal cancer, bladder cancer, and melanoma. . Further investigation revealed that the effect of inosine relies on the adenosine A2A receptor expression and required costimulation specifically in T lymphocytes.[63]

Recently, the application of omics platforms has been used as a powerful tool to characterize anticancer relevant bacterial isolates.Claudia Grajeda-Iglesias et al performed metabolomics analysis of Ackermania by chromatographic and mass spectrometric methods. The results revealed that Ackermania improved anticancer immune surveillance by enhancing polyamines, SCFA, and multiple bile acids. Lipidomics analysis was performed to evaluate the influence of probiotics in host physiology. A study was conducted to investigate alterations in lipid composition in host and bacteria and found that differences in phosphatidylglycerol and phosphatidylcholine contents could impact nematode host physiology.[64] In addition, quantitative proteomics approach was used when cultivating the probiotic *strain Enterococcus durans* with prebiotic fructo-oligosacchrides, and the result showed that *Enterococcus durans* was stimulated to produce clinically important cancer therapeutics, L-asparaginase and arginine deiminase.[65]

Dietary intervention and life style

As gut microorganisms contribute to food digestion, the association between diet and the microbiota has been investigated for several years at different levels of resolution.[66,67] Indeed, the sequential host digestion and nutrient extraction is intimately involved with different microbial communities, with the gut microbiota playing the largest role.[68] On the one hand, gut microbiota releases a mass of substances that the host is unable to digest, thus altering nutritional availability of food. On the other hand, both short- and long-term dietary change can influence the microbial transcriptomic and metabolomic profiles, pariicularlly for infant nutrition that may have life-long consequences through microbial modulation of the immune system. For example, high fat diets are related to substantial changes in the colonic microbiota composition, including reductions in both Gram positive (e.g., *Bifidobacterium spp.*) and Gram negative bacteria (e.g., *Bacteroides*).[69] They may also increase quantities of pro-inflammatory gut microbes by stimulating the formation of taurine-conjugated bile acids that promotes growth of these pathogens.[70] Moreover, when humans were fed with plant-based diet, the bacterial taxa with fiber-degrading capacity increased accordingly. Mardinoglu et al. observed that reduction of carbohydrate (including fiber) intake dramatically decreased the abundance of fiber-degrading bacteria within 24 h in a human cohort study, while the abundance of *Lactococcus, Eggerrthella, and Streptococcus* increased, resulting in reduced levels of SCFAs.[71] Further, based on data from 185 prospective studies and 58 clinical trials with 4635 adult participants comparing the highest dietary fiber consumers with the lowest, Reynolds and colleagues found a 15-30% lower rate in all-cause and cardiovascular-related mortality, in addition to a lower incidence and mortality in cardiovascular

diseases, type 2 diabetes, and colorectal, and breast cancer.[72] Spencer et al presented the data that consumption of a high-fiber diet was associated with higher gut microbiome diversity and better response to anti-PD-1 immunotherapy in melanoma patients at a presscast in advance of the AACR Annual Meeting 2019 (Abstract 2838/24). Of the 46 patients who received anti-PD-1 treatment, they found that patients who consumed a high-fiber diet were about five times more responsive to immunotherapy than those with a low fiber diet. Except as boosting the efficacy of PD-1 inhibitor, diet intervention has also been explored for the property of reducing the incidence of treatment-emergent adverse events. MicrSoy-20(MS-20), fermented soybean extract, has proven to be a chemotherapy adjuvant to ameliorate chemo-associated fatigue and appetite loss in cancer patients by remodeling human gut ecosystem and restoring immunity. Therefore, a randomized-controlled trial was performed to evaluate the safety and potential clinical outcomes of NSCLC patients who were treated with a combination of anti-PD-1 antibody and MS-20 at the Taipei Medical University Hospital from Taiwan (NCT04909034).

In addition to diet intervention, increasing data support that life style is crucial determinant for the gut microbial composition, whose alterations induce local and systemic immune response, thereby contributing to cancer development. As indicated from substantial evidence, consumption of alcohol is related to increased risk of colorectal cancer in a dose-dependent manner, and abstinence from alcohol has been shown to restore gut barrier integrity in humans.[73] Moreover, Long-term ethanol exposure has shown to reduce the abundance of butyrate-producing taxa from the *Clostridiales* order, like *Faecalibacterium prausnitzii, Coprococcus eutactus,* etc.[74] M.D. Anderson Cancer Center sponsored a randomized clinical trial conducted in patients with stage II or III breast cancer undergoing radiation therapy, investigating whether an integrative oncology (making changes in lifestyle and behavior) program can improve cancer-related outcomes of these patients. The intervention in lifestyle and behavior including but not limited to dietary recommendations, physical activity, and control of environmental contaminants, which are likely to modify the efficacy of cancer therapy and improve the quality of life of patients. The researchers not only measure disease-free survival and changes in biological pathways as the primary outcomes, but also collect longitudinal gut and oral microbiome samples and analyses a battery of questionnaires for examining the influence of microbiome changes in breast cancer patients (NCT02079662). Additionally, an observational study was designed to evaluate how the microbiome interacts with efficacy of immune checkpoint inhibitors in patients with advanced cancer (melanoma, renal and lung cancer), with the investigators collecting saliva and fecal samples for microbiome analysis, and linking it to therapy response by examining blood and tumor samples (NCT04107168). They further correlate the microbiome findings with preexisting patient behavioral characteristics, including diet, smoking history BMI, and use of antibiotics.

Conclusion

There is compelling evidence from the preclinical and clinical studies suggesting that the gut microbiome play a major role in affecting host immunity and therapeutic response in cancer, either through a local presence within the tumor microenvironment or via the systemic antiviral immune responses. The latter is probably the reason why the gut microbiota is capable of regulating the host response to conventional chemotherapeutic agents and immunotherapy, ultimately having various impacts on patient outcomes.

Outstanding questions

Although significant progresses have been made regarding the role of the gut microbiota in response to cancer therapy, there remains a great deal to explore in the future studies. For example, patient populations varied substantially in different clinical trials, meanwhile there is little consistency across these studies regarding the specific bacteria recognition or effect on immunotherapy responses. First, according to the findings, extrinsic factors such as diet, medications (including antibiotics, prebiotics and probiotics), lifestyle, mental health and other environmental factors are also essential in modulating the microbial composition and overall immune status, thereby affecting cancer immunotherapy responses or toxicities. Second, variations in sequencing methodologies (16S rRNA sequencing versus whole-genomic sequencing) and selection of different reference databases also led to the results inconsistency to some extent. This calls the potential need for standardization of microbiome profiling techniques at every level of analysis, and a systematic study integrating gut transcriptome, proteome, and metabolome for a comprehensive understanding of the relevant microbiota.

In the future, there is still tremendous to explore with regard to the mechanisms into these complex interactions as well as the specific microorganisms which play the most crucial role in mediating antitumor responses and overall cancer developments. Considering all these internal and external factors, multidimensional strategies need to be adopted to optimize the better state of this complex ecosystem and effectively improve therapeutic efficacy.

Search strategy and selection criteria

Data for this Review were identified by searches of PubMed and Google Scholar, references from relevant articles using the search terms "gut microbiome and host

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immunity", "gut microbiome and checkpoint inhibitor", "fecal microbiota transplantation and immunotherapy", "probiotics and immunotherapy", "prebiotics and immunotherapy", "diet and Immunotherapy". Most of references are articles published between 2011 and 2021, and few of them are reviews to explain well-known concepts.

References

1   Morad G, Helmink BA, Sharma P, et al. Hallmarks of response, resistance, and toxicity to immune checkpoint blockade. *Cell*. 2021;184(21):5309–5337.

2   Arbour KC, Riely GJ. Systemic therapy for locally advanced and metastatic non-small cell lung cancer: a review. *JAMA*. 2019;322 (8):764–774.

3   Thaiss CA, Zmora N, Levy M, et al. The microbiome and innate immunity. *Nature*. 2016;535(7610):65–74.

4   Fukata M, Arditi M. The role of pattern recognition receptors in intestinal inflammation. *Mucosal Immunol*. 2013;6(3):451–463.

5   Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. 2010;140(6):805–820.

6   Pinacchio C, Scagnolari C, Iebba V, et al. High abundance of genus Prevotella is associated with dysregulation of IFN-I and T cell response in HIV-1-infected patients. *AIDS*. 2020;34 (10):1467–1473.

7   Dalod M, Chelbi R, Malissen B, et al. Dendritic cell maturation: functional specialization through signaling specificity and transcriptional programming. *Embo J*. 2014;33(10):1104–1116.

8   Abt MC, Osborne LC, Monticelli LA, et al. Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity*. 2012;37(1):158–170.

9   Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. *Cell*. 2010;140(6):845–858.

10 Ivanov II, Frutos Rde L, Manel N, et al. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe*. 2008;4(4):337–349.

11 Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009;139(3):485–498.

12 Strugnell RA, Wijburg OL. The role of secretory antibodies in infection immunity. *Nat Rev Microbiol*. 2010;8(9):656–667.

13 Brandtzaeg P. Secretory IgA: designed for anti-microbial defense. *Front Immunol*. 2013;4:222.

14 Corthésy B. Multi-faceted functions of secretory IgA at mucosal surfaces. *Front Immunol*. 2013;4:185.

15 Fagarasan S, Kawamoto S, Kanagawa O, et al. Adaptive immune regulation in the gut: T cell-dependent and T cell-independent IgA synthesis. *Annu Rev Immunol*. 2010;28:243–273.

16 Blutt SE, Conner ME. The gastrointestinal frontier: IgA and viruses. *Front Immunol*. 2013;4:402.

17 Pabst O, Cerovic V, Hornef M. Secretory IgA in the coordination of establishment and maintenance of the microbiota. *Trends Immunol*. 2016;37(5):287–296.

18 Moor K, Diard M, Sellin M E, et al. High-avidity IgA protects the intestine by enchaining growing bacteria. *Nature*. 2017;544 (7651):498–502.

19 Fukuda S, Toh H, Hase K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*. 2011;469(7331):543–547.

20 Donohoe DR, Garge N, Zhang X, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab*. 2011;13(5):517–526.

21 Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*. 2018;359(6371):91–97.

22 Paulos CM, Wrzesinski C, Kaiser A, et al. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling. *J Clin Invest*. 2007;117(8):2197–2204.

23 Balachandran VP, Łuksza M, Zhao J N, et al. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. *Nature*. 2017;551(7681):512–516.

24 Gurav A, Sivaprakasam S, Bhutia YD, et al. Slc5a8, a Na+-coupled high-affinity transporter for short-chain fatty acids, is a conditional tumour suppressor in colon that protects against colitis and colon cancer under low-fibre dietary conditions. *Biochem J*. 2015;469 (2):267–278.

25 Trompette A, Gollwitzer ES, Yadava K, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med*. 2014;20(2):159–166.

26 Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504(7480):451–455.

27 White CA, Pone EJ, Lam T, et al. Histone deacetylase inhibitors upregulate B cell microRNAs that silence AID and Blimp-1 expression for epigenetic modulation of antibody and autoantibody responses. *J Immunol*. 2014;193(12):5933–5950.

28 Zhang Z, Tang H, Chen P, et al. Demystifying the manipulation of host immunity, metabolism, and extraintestinal tumors by the gut microbiome. *Signal Transduct Target Ther*. 2019;4:41.

29 Mcdonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis*. 2018;66(7):e1–e48.

30 Van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med*. 2013;368(5):407–415.

31 Khoruts A, Staley C, Sadowsky MJ. Faecal microbiota transplantation for Clostridioides difficile: mechanisms and pharmacology. *Nat Rev Gastroenterol Hepatol*. 2021;18(1):67–80.

32 Cheng YW, Phelps E, Ganapini V, et al. Fecal microbiota transplantation for the treatment of recurrent and severe Clostridium difficile infection in solid organ transplant recipients: a multicenter experience. *Am J Transplant*. 2019;19(2):501–511.

33 Gopalakrishnan V, Spencer C N, Nezi L, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science*. 2018;359(6371):97–103.

34 Matson V, Fessler J, Bao R, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science*. 2018;359(6371):104–108.

35 Riquelme E, Zhang Y, Zhang L, et al. Tumor microbiome diversity and composition influence pancreatic cancer outcomes. *Cell*. 2019;178(4):795–806.e12.

36 Baruch EN, Youngster I, Ben-Betzalel G, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science*. 2021;371(6529):602–609.

37 Davar D, Dzutsev AK, Mcculloch JA, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science*. 2021;371(6529):595–602.

38 Youngster I, Russell GH, Pindar C, et al. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. *JAMA*. 2014;312(17):1772–1778.

39 Angelberger S, Reinisch W, Makristathis A, et al. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. *Am J Gastroenterol*. 2013;108 (10):1620–1630.

40 Defilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant E. coli bacteremia transmitted by fecal microbiota transplant. *N Engl J Med*. 2019;381(21):2043–2050.

41 Kelly CR, Ihunnah C, Fischer M, et al. Fecal microbiota transplant for treatment of Clostridium difficile infection in immunocompromised patients. *Am J Gastroenterol*. 2014;109(7):1065–1071.

42 Wang S, Xu M, Wang W, et al. systematic review: adverse events of fecal microbiota transplantation. *PLoS One*. 2016;11(8):e0161174.

43 Zaretsky JM, Garcia-Diaz A, Shin DS, et al. Mutations associated with acquired resistance to PD-1 blockade in Melanoma. *N Engl J Med*. 2016;375(9):819–829.

44 Jenkins RW, Barbie DA, Flaherty KT. Mechanisms of resistance to immune checkpoint inhibitors. *Br J Cancer*. 2018;118(1):9–16.

45 Sharma P, Hu-Lieskovan S, Wargo JA, et al. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell*. 2017;168 (4):707–723.

46 Shin DS, Zaretsky JM, Escuin-Ordinas H, et al. Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. *Cancer Discov*. 2017;7(2):188–201.

47 Spranger S, Gajewski TF. Tumor-intrinsic oncogene pathways mediating immune avoidance. *Oncoimmunology*. 2016;5(3):e1086862.

48 Spranger S, Gajewski TF. Impact of oncogenic pathways on evasion of antitumour immune responses. *Nat Rev Cancer*. 2018;18 (3):139–147.

49 Lim YW, Chen-Harris H, Mayba O, et al. Germline genetic polymorphisms influence tumor gene expression and immune cell infiltration. *Proc Natl Acad Sci USA*. 2018;115(50):E11701–E11710.

50 Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506–514.

51 Martín R, Langella P. emerging health concepts in the probiotics field: streamlining the definitions. *Front Microbiol*. 2019;10:1047.

52 Hibberd AA, Lyra A, Ouwehand AC, et al. Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. *BMJ Open Gastroenterol*. 2017;4(1):e000145.

53 Zaharuddin L, Mokhtar NM, Muhammad Nawawi KN, et al. A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterol*. 2019;19(1):131.

54 Vétizou M, Pitt JM, Daillère R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*. 2015;350 (6264):1079–1084.

55 Chaput N, Lepage P, Coutzac C, et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol*. 2017;28(6):1368–1379.

56 Atarashi K, Tanoue T, Shima T, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science*. 2011;331 (6015):337–341.

57 Raman M, Ambalam P, Kondepudi KK, et al. Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. *Gut Microbes*. 2013;4(3):181–192.

58 Mcloughlin RF, Berthon BS, Jensen ME, et al. Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: a systematic review and meta-analysis. *Am J Clin Nutr*. 2017;106 (3):930–945.

59 Taper HS, Roberfroid MB. Possible adjuvant cancer therapy by two prebiotics—inulin or oligofructose. *In Vivo*. 2005;19(1):201–204.

60 Looijer-Van Langen MA, Dieleman LA. Prebiotics in chronic intestinal inflammation. *Inflamm Bowel Dis*. 2009;15(3):454–462.

61 Herfel TM, Jacobi SK, Lin X, et al. Polydextrose enrichment of infant formula demonstrates prebiotic characteristics by altering intestinal microbiota, organic acid concentrations, and cytokine expression in suckling piglets. *J Nutr*. 2011;141(12):2139–2145.

62 Cani PD, Possemiers S, Van De Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut*. 2009;58(8):1091–1103.

63 Mager LF, Burkhard R, Pett N, et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science*. 2020;369(6510):1481–1489.

64 Schifano E, Cicalini I, Pieragostino D, et al. In vitro and in vivo lipidomics as a tool for probiotics evaluation. *Appl Microbiol Biotechnol*. 2020;104(20):8937–8948.

65 Comerlato CB, Zhang X, Walker K, et al. Comparative proteomic analysis reveals metabolic variability of probiotic Enterococcus durans during aerobic and anaerobic cultivation. *J Proteomics*. 2020;220:103764.

66 Flint HJ, Scott KP, Louis P, et al. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol*. 2012;9 (10):577–589.

67 Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature*. 2016;535(7610):56–64.

68 Ley RE, Hamady M, Lozupone C, et al. Evolution of mammals and their gut microbes. *Science*. 2008;320(5883):1647–1651.

69 Nava GM, Carbonero F, Ou J, et al. Hydrogenotrophic microbiota distinguish native Africans from African and European Americans. *Environ Microbiol Rep*. 2012;4(3):307–315.

70 Devkota S, Wang Y, Musch MW, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/-mice. *Nature*. 2012;487(7405):104–108.

71 Mardinoglu A, Wu H, Bjornson E, et al. An integrated understanding of the rapid metabolic benefits of a carbohydrate-restricted diet on hepatic steatosis in humans. *Cell Metab*. 2018;27(3):559–571.e5.

72 Reynolds A, Mann J, Cummings J, et al. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet*. 2019;393(10170):434–445.

73 Leclercq S, Matamoros S, Cani P D, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc Natl Acad Sci USA*. 2014;111(42):E4485–E4493.

74 Dubinkina VB, Tyakht AV, Odintsova VY, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome*. 2017;5(1):141.