Association between the gut microbiota and patient responses to cancer immune checkpoint inhibitors (Review)

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Abstract. Studies are increasingly investigating the association between the gut microbiota and the outcomes of immunotherapy in patients with cancer. Notably, certain studies have demonstrated that the gut microbiota serves a key role in regulating a patient's response to immunotherapy. In the present review, the potential associations between the gut microbiota, and cancer, host immunity and cancer immunotherapy are reviewed. Furthermore, the effects of fecal microbiota transplantation, antibiotics, probiotics, prebiotics, synbiotics, components of traditional Chinese medicine and various lifestyle factors on the gut microbiota and cancer immunotherapy outcomes are discussed. Certain dominant bacterial groups in the context of cancer immunotherapy and certain effective methods for optimizing immunotherapy by regulating the gut microbiota have been identified. Further investigation may enable the rapid conversion of these discoveries into practical products and clinically applicable methods.

Contents

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Abbreviations: CTLA-4, cytotoxic T-lymphocyte antigen-4; FMT, fecal microbiota transplantation; GQD, Gegen Qinlian decoction; ICIs, immune checkpoint inhibitors; NSCLC, non-small-cell lung cancer; OS, overall survival; PD-1, programmed death protein 1; PD-L1, programmed death ligand 1; PFS, progression-free survival; SPF, specific pathogen-free; TCRs, T-cell receptors; TLR, toll-like receptor

Key words: antibiotics, CTLA-4, FMT, PD-1, PD-L1, traditional Chinese medicine

1. Introduction

Cancer is a leading cause of mortality worldwide. In 2015, 4.292 million new cancer cases and 2.814 million cancer-related mortalities were reported in China (1). Cancer immunotherapy is one of several treatments for various types of cancer, and has yielded great success when applied to the treatment of certain hematological and solid malignancies (2-4). Currently available immune-targeted cancer therapies include Toll-like receptor (TLR) agonists, vaccines, immune checkpoint inhibitors (ICIs) and adoptive T-cell therapy.

ICIs serve a key role in the treatment of several cancer types. This class of therapy is exemplified by monoclonal antibodies that block the programmed death protein 1/programmed death ligand 1 (PD-1/PD-L1) axis and the cytotoxic T-lymphocyte antigen-4 (CTLA-4) cell-surface receptor (5,6). ICIs restore the immune system's ability to target and kill cancer cells by inhibiting suppressive interactions between T-cell receptors and homologous ligands on cancer cells (7). The present review investigated the increasing volume of research regarding gut microbiota as a regulator of the effects of immunotherapy in patients with cancer.

2. Gut microbiota and cancer

The gut microbiota is closely associated with cancer via its ability to influence malignancy through a variety of direct and indirect mechanisms (8). For example, certain products of the gut microbiota may directly promote cancer growth, including metabolites produced by intestinal microorganisms that directly induce oncogenic mutations in the host (9). Furthermore, *Escherichia coli* strains that harbor the *pks* virulence gene island may produce the toxin colibactin, which has been shown to cause genetic damage and subsequent colorectal cell malignancy when injected into cultured human intestinal stem cells *in vitro* (9).

Intestinal bacteria may not directly promote tumorigenesis and cancer development, but instead may interact with the immune system to indirectly promote malignancy (8). A defective immune response may also lead to an increase in the abundance of certain bacterial genera, and the resulting

immune response triggers signaling pathways that lead to the transcription of oncogenes (8). Furthermore, the gut microbiota may indirectly promote cancer by inducing inflammation or immunosuppression (10,11). Finally, changes in the composition of the gut microbiota are closely associated with the development of various malignancies, including gastric cancer, colorectal cancer, liver cancer, pancreatic cancer, breast cancer and melanoma (12).

3. Gut microbiota and immunity

The gut microbiota affects various aspects of host immunity (Fig. 1). In particular, the interaction between the gut microbiota and the intestinal mucosal immune system is considered a key factor in the maintenance of mucosal homeostasis. The intestinal mucosal immune system has unique structures and functions that are relatively independent of systemic immunity (11). For example, it can effectively inhibit bacterial adhesion and colonization in intestinal epithelial cells and can sequester bacteria and harmful antigens within the body (6). The gut microbiota exerts a wide range of effects on the intestinal mucosal immune system of the host (13). For example, *Bacteroides fragilis* may induce naïve $CD4^+$ naive T-cells to differentiate into regulatory T-cells (Treg cells) that secrete large quantities of anti-inflammatory cytokines (e.g., IL-10) (14). Furthermore, Cebula *et al* (15) reported that Treg cells in the colon may recognize antigenic substances associated with the bacterial genera *Clostridium* and *Bacteroides* (15,16).

The gut microbiota and systemic immunity are also closely associated through several mechanisms. To begin with, small molecular substances produced by the gut microbiota may enter the blood circulation and thereby affect immune responses in distant organs. Furthermore, the gut microbiota shares a mucosal network and therefore a common mucosal immune system with mucosal tissues throughout the body. Additionally, extra-intestinal diseases may be caused by changes in the immune response induced by signals produced by the gut microbiota and recognized by TLRs on host immune cells. Furthermore, the gut microbiota regulates the development of systemic immune cells (6). Therefore, a gut microbiota that contains a higher proportion of beneficial bacteria is associated with a more completely developed immune system that is better adapted to the external environment (6). In summary, the gut microbial community has profound effects on the local and systemic immune systems. Furthermore, the immune system may also effect change in the gut microbiome (11).

4. Gut microbiota and immunotherapy

Antitumor mechanism of ICIs. Immunotherapy targets regulatory T-cell pathways and thus enhances the anticancer immune response (17). To date, applications of this novel cancer therapeutic modality have been proven effective in a range of clinical contexts. Certain patients can obtain long-lasting clinical effects from immunotherapy and may even achieve good long-term outcomes in the absence of a cancer burden. Furthermore, immunotherapy has afforded researchers an improved understanding of the human immune response in the cancer microenvironment (18).

Host recognition and killing of cancer cells relies on T-cell-mediated cellular immunity. T-cells bind via their T-cell receptors (TCRs) to specific antigens associated with major histocompatibility complex (MHC) molecules expressed on the surfaces of cancer cells. These interactions of TCRs with MHC molecules are controlled by a series of immune checkpoints, co-stimulatory or co-suppressive signals that lead to the activation or suppression of T-cells. CTLA-4, PD-1 and PD-L1 are synergistic inhibitory molecules that suppress immune responses and thus prevent the pathological targeting of self-antigens (i.e., autoimmune disease). The PD-1/PD-L1 axis serves an important role in immune tolerance via the transmission of co-suppressive signals that may suppress the immune activity of T-cells and enable cancer cells to escape host immunity (18). To date, monoclonal antibodies targeting the checkpoints CTLA-4 and the PD-1/PD-L1 axis have yielded significant successes in the field of clinical immunotherapy.

Gut microbiota and CTLA-4. The effects of the gut microbiota on the efficacy and toxicity of anti-CTLA-4 therapy have been previously investigated (Table I). In a study on patients with metastatic melanoma who were treated with a CTLA-4-targeting antibody, those whose gut microbiota profiles were rich in species of the Enterobacteriaceae family, including *Enterobacter faecalis*, and other Firmicutes spp., achieved longer progression-free survival (PFS) and overall survival (OS) times. However, similar outcomes were not observed in patients with a microbiota rich in *Bacteroides* spp. (19). Another study demonstrated that germ-free (GF) or antibiotic-treated mice do not respond as well to CTLA-4-inhibiting antibodies as do specific pathogen-free (SPF) mice (20). An analysis of bacterial isolates revealed that the presence of the species *B. polymorpha*, *B. fragilis* and *Burkholderia cepacia* was closely associated with the efficacy of CTLA-4-targeting therapy and associated with fewer adverse treatment effects (21). Another study demonstrated that oral supplementation with *Bacteroides* spp. could restore the efficacy of immunotherapy by increasing the number of mature dendritic cells in a tumor and enhancing the Th1 response in the draining lymph nodes. Taken together, these findings indicated that the effects and toxicities associated with anti-CTLA-4 treatment may be influenced by the profile and concentration of intestinal bacteria (21,22).

Notably, antibiotic treatment was demonstrated to decrease the effectiveness of anti-IL-10/CpG oligonucleotide immunotherapy in mouse models of MC38 cell line-induced colon cancer and subcutaneous B16 cell line-induced melanoma. It is possible that this antibiotic therapy decreased the gut microbiota load and the population of monocytes that produce pro-inflammatory cytokines (23,24). The results of a recent study also suggested that patients who used antibiotics 30 days before immunotherapy had a significantly shorter PFS time than those who did not receive antibiotic therapy (25). Regarding toxicity, studies of the gut microbiota in patients receiving anti-CTLA-4 treatment revealed an increased abundance of *Faecalibacterium* spp. and decreased abundance of *Bacteroides* spp., as well as an increased risk of colitis (11,21,26).

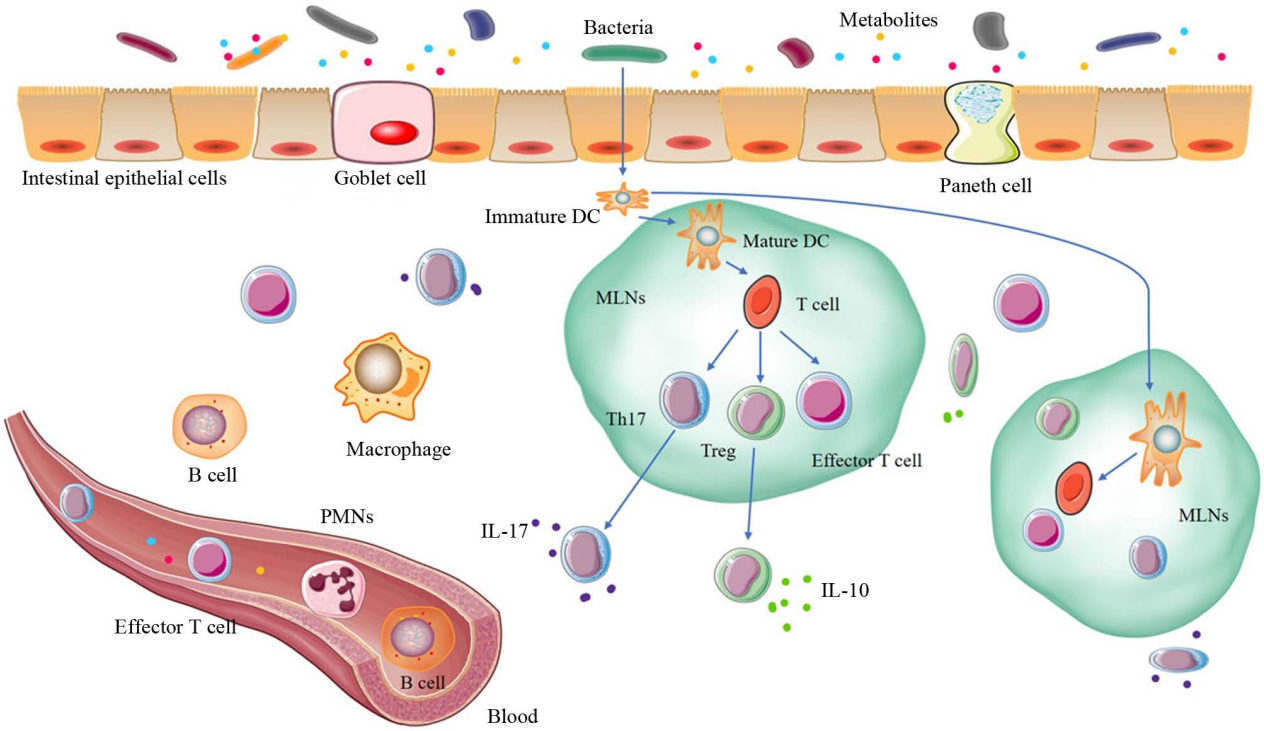

Figure 1. Gut microbiota and immunity. The intestinal mucosa is a single epithelial cell layer, composed of IECs and intraepithelial lymphocytes; the goblet and pane cells are located among the IECs. The bacteria and bacterial metabolites can activate DCs to transfer to MLNs. Mature DCs activate T cells to differentiate into effector T cells, Tregs or Th17 cells, which can transfer them back to the intestinal mucosa and systemic circulation. In the local immune response, Tregs secrete IL-10 and create a local anti-inflammatory cytokine environment. Th17 cells secrete IL-17 cytokines, and IL-17 can induce paneth cells to produce antimicrobial peptides and can recruit PMNs from the bloodstream. Certain bacterial metabolites can enter the blood directly, further changing the immune system. B cells and T cells can enter the systemic circulation to promote the immune response to the same antigen at a distance (11,79). IECs, intestinal epithelial cells; DCs, dendritic cells; MLNs, mesenteric lymph nodes; PMNs, polymorphonuclear leukocytes; IL, interleukin; Treg, regulatory T cells; Th17, T-helper cell 17.

Gut microbiota and PD-1/PD-L1. Previous studies have also investigated the effects of gut microbiota on the therapeutic effects and toxicities associated with treatments targeting the PD-1/PD-L1 axis (Table I) (19,27). Sivan *et al* (28) observed that different responses to anticancer immunotherapy in a mouse model of melanoma were associated with differences in commensal gut microbiota profiles. Furthermore, these differences disappeared following cohabitation or fecal transplantation between groups (23). A 16S rRNA sequence analysis revealed that *Bifidobacterium* spp. may enhance the effectiveness of anticancer immunotherapy. Specifically, an orally administered *Bifidobacterium* supplement was shown to improve the level of cancer control afforded by PD-L1-specific antibody treatment when compared with the probiotic-free antibody treatment. Furthermore, combined treatment with the PD-L1-targeted therapy and *Bifidobacterium* nearly eliminated tumor growth (23). Mechanistically, this combination therapy appears to promote the functions of dendritic cells, which leads to an increase and accumulation of $CD8^+$ T-cells in the cancer microenvironment and an enhanced anticancer effect (23).

In another study, the response to anti-PD-L1 therapy was shown to depend on the composition of the gut microbiota, with *Bifidobacterium* spp. found to be highly associated with an effective anti-PD-L1 response (28). In mouse experiments, supplementation with *Bifidobacterium* spp. restored the efficacy of PD-L1-targeted immunotherapy by promoting an increase in the $CD8^+$/IFN-γ^+ T-cell population within tumors (23). Additional studies have demonstrated that microbiota composition is predictive of the response statuses of patients receiving anti-PD-1/PD-L1 therapies for solid epithelial cancers (29-31).

Gopalakrishnan *et al* (29) reported that *Faecalibacterium* were abundant in the intestinal microbiota of patients with melanoma who responded to anti-PD-L1 treatment (29), while *Bacteroides thetaiotaomicron*, *Escherichia coli* and *Anaerotruncus colihominis* were abundant in patients who achieved poor treatment effects. Notably, transplantation of the fecal microbiota from a human patient who had responded well to anti-PD-L1 treatment into GF mice improved the efficacy of this immunotherapy in mice. The efficacy of this anticancer immunotherapy may have bene positively associated with the numbers of mature dendritic cells (DCs) and IFN-γ^+, $CD8^+$ and/or $CD4^+$ anticancer T-cells in a tumor, and negatively associated with the number of $CD4^+$, $FoxP3^+$ Treg cells in a tumor (29,30).

Notably, a study on the gut microbiota of 25 patients with melanoma who had received anti-PD-1 therapy revealed significant differences in the diversity and composition of patients' microbiota, with the stool of patients who responded well to therapy containing large concentrations of *Bacilli* (29). However, although these strains were positively correlated with PFS, they were also associated with an increased ultimate risk of cancer recurrence and growth (29). Other studies have analyzed baseline stool samples collected from immunotherapy-naïve patients with metastatic melanoma

Table I. Summary of featured microorganisms in cancer immunotherapy studies.

Immunotherapy	Tumor	Model	Bacteria	Main findings	(Refs.)
CTLA-4 mAb	Metastatic melanoma	Human	*Faecalibacterium*, other *Firmicutes*	Enriched with *Faecalibacterium* and other *Firmicutes* is associated with ICI responders	(19)
CTLA-4 mAb	Melanoma	Human, mouse	*Bacteroides thetaiotaomicron*, *B. fragilis*	Increased level of *Bacteroides thetaiotaomicron* or *B. fragilis* was associated with the efficacy of CTLA-4 blockade	(20)
PD-1 mAb	Metastatic melanoma	Human	*Bifidobacterium longum, Collinsella aerofaciens, Enterococcus faecium*	Bacterial species more abundant in responders included *Bifidobacterium longum*, *Collinsella aerofaciens* and *Enterococcus faecium*	(23)
CTLA-4 mAb, PD-1 mAb	Metastatic melanoma	Human	*Bacteroides caccae*	ICI responders were enriched for *Bacteroides caccae*	(26)
PD-L1 mAb	Melanoma	Mouse	*Bifidobacterium*	Combination of *Bifidobacterium* and PD-L1 abolished tumor outgrowth	(28)
PD-1 mAb	Melanoma	Human	*Ruminococcaceae*	Increased the level of *Ruminococcaceae* family in responding patients	(29)
PD-1 mAb	Melanoma	Mouse	*Akkermansia muciniphila*	Relative abundance of *Akkermansia muciniphila* in ICI clinical responders	(40)
PD-1 mAb	NSCLC, GC	Human	*Ruminococcaceae*	Higher alpha diversity and *Ruminococcaceae* levels in ICI responders	(80)
PD-1 mAb	RCC	Human	*Roseburia spp, Faecalibacterium spp*	Increased the level of *Roseburia* and *Faecalibacterium spp* in ICI responders	(27)

CTLA-4, cytotoxic T-lymphocyte antigen-4; PD-1, programmed death protein 1; ICI, immune checkpoint inhibitor; GC, gastric cancer; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; RCC, renal cell carcinoma.

and identified a significant association between patient gut microbiota and their clinical response to immunotherapy (23). In particular, *Bifidobacterium longum*, *Collinsella aerogenes* and *Enterococcus faecium* were more abundant in the gut microbiota of patients who responded well to treatment (23). Regarding toxicity, previous studies have reported that antibodies targeting PD-1 and PD-L1 may cause thyroid dysfunction and pneumonia (32,33).

In summary, it is evident that there is an association between patient gut microbiota and their response to anticancer immunotherapy. Specifically, the abundance and range of organisms in the gut microbiota may aid or hinder cancer immunotherapies, in terms of efficacy and side effects. These observations strongly support the integration of microbial therapies into anticancer immunotherapy strategies with the aim of improving the efficacy of immunotherapy while decreasing toxicity. Therefore, the regulation of the gut microbiota to ensure more effective anticancer immunotherapy within a wider therapeutic window (i.e., with fewer toxic effects) has become a promising research direction.

5. Improving immunotherapy by regulating the gut microbiota

As demonstrated earlier, the gut microbiota intimately affects the outcomes of cancer immunotherapy. Consequently, it has been demonstrated that regulation of the gut microbiota may improve the efficacy and decrease the adverse effects of cancer immunotherapy (34-36). Despite this, associated improvements in cancer treatment outcomes and prognoses remain very limited (37). The methods used to regulate the gut microbiota and potentially optimize anticancer immunity are therefore discussed in the following sections.

Fecal microbiota transplantation (FMT). FMT is defined as the transplantation of the gut microbiota from a healthy donor, in the form of diluted fecal material, into a patient via the

upper or lower digestive tract with the intent to restore intestinal microbial diversity (38,39). FMT may regulate the effects of anticancer immunotherapy by rebuilding the gut microbiota and improving bile acid metabolism (12). Studies of FMT in a mouse model of MCA-205 sarcoma revealed that mice treated with anti-PD-1 therapy and an effective FMT from patients who responded well to PD-1 therapy exhibited significantly delayed cancer growth, while no delay in tumor growth was observed in mice that received FMT from patients who did not respond to PD-1 treatment (40). Wang *et al* (41) reported the first case of successful treatment of ICI-associated colitis with FMT (41), using a method that reconstructed the gut microbiota and led to a relative increase in the proportion of Treg cells in the intestinal mucosa (6). Although cancer in GF or antibiotic-treated mice responds poorly to ICI therapy, mice dosed with FMT from patients who have responded successfully to immunotherapy responded more positively to the same ICI therapies. Analysis of these gut microbiota in FMT revealed large abundances of the genera *Bacteroides* (42), *Burkholderia* (42), *Akkermansia* (43), *Faecalis* (44) and *Clostridium* (45). FMT has been used widely and very successfully to treat refractory *Clostridium* infections (46), which is encouraging with respect to the treatment of other diseases.

There are three important points to note prior to administering FMT. To begin with, bacterial species must be accurately isolated and screened to ensure that they may improve the efficacy of anticancer immunotherapy in the host. Furthermore, harmful bacteria, viruses and parasites must be removed. Additionally, attention should be paid to the isolation and cultivation of less abundant but important microorganisms (11).

The clinical value of FMT has been demonstrated in certain studies, but data specific to the field of cancer immunotherapy remain limited and are primarily derived from animal models (38,47). The fecal bacterial composition and pathogenicity are unknown, and the safety of FMT remains controversial. Certain studies have reported certain minor adverse events, including low-grade fever, constipation and diarrhea following FMT. Serious side effects are relatively rare, including infection and/or sepsis, pneumonia and complications of endoscopy (38,47,48). Furthermore, FMT is an emerging treatment without a long history of use, and therefore long-term safety surveys are lacking (12). Further investigation is required in this area, and several clinical trials are underway (Table II) (49).

Antibiotics. It has been demonstrated that the use of antibiotics may reduce the benefits of anticancer immunotherapy (50,51). For example, Elkrief *et al* (25) studied patients with advanced melanoma who were treated with anti-PD-1 or anti-CTLA-4 monoclonal antibody therapies alone or in combination with chemotherapy and had or had not received antibiotics within 30 days after the start of immunotherapy. Notably, the authors observed that antibiotic treatment adversely affected the patient prognoses (25).

In another study it was determined that the use of antibiotics, and particularly broad-spectrum antibiotics, affected the prognosis of patients treated with ICIs (52). Specifically, Ahmed *et al* (52) investigated whether the use of antibiotics during immunotherapy would alter the efficacy of the latter treatment in a sample of 60 patients with advanced cancer, including 17 who had received antibiotics for a microbial infection within 2 weeks before or after the start of immunotherapy; the results demonstrated that immunotherapy was less effective in patients who had received systemic antibiotics than in those who had not received antibiotics, and reduced immunotherapeutic efficacy and shorter OS duration were observed in patients who used broad-spectrum antibiotics (those effective against both Gram-positive and negative bacteria, including aerobic and anaerobic bacteria) compared with those who used narrow-spectrum antibiotics (those effective only against Gram-positive bacteria) (52).

Other researchers retrospectively analyzed 90 patients with non-small-cell lung cancer who were treated with nivolumab, 13 of whom had also received antibiotic therapy; although the researchers observed a negative effect of antibiotic use on the outcome of immunotherapy, they did not find a statistically significant correlation between survival and antibiotic use (53). They suggested that the interval between antibiotic treatment and nivolumab treatment initiation may play an important role in the microbiota-influenced response to treatment, as the composition of a patient's gut microbiota changes dramatically after antibiotic therapy is stopped (54).

Antibiotic use within 2-3 months before or after the start of immunotherapy was also reported to be associated with reduced PFS duration and OS, which may be related to the loss of homeostasis in the gut microbiota (49). Three factors can explain the gut microbiota changes caused by antibiotics: The loss of microorganisms, the direct effects of antibiotics on host tissues, and the effects of antibiotics on the remaining resistant microorganisms. Normal microbial depletion leads to the suppression of all aspects of immunity, whereas the direct effects of antibiotics on the host tissues and the actions of antibiotic-resistant microorganisms inhibit mitochondrial gene expression and reduce the number of active mitochondria, leading to epithelial cell death (55). These actions of antibiotics may reduce the effectiveness of anti-cancer immunotherapy, and, together with the observations detailed above, highlight that antibiotic therapy should be minimized before and during cancer immunotherapy.

Probiotics, prebiotics and symbiotics. Probiotics are active microorganisms that may maintain health by improving or restoring the intestinal flora (56). Certain combinations of probiotics may enhance the immune responses of patients by changing the gut microbiota (57). It has been demonstrated that significantly higher proportions of patients treated with probiotics had normal ratios of health-related blood biomarkers, namely $CD3^+$, $CD4^+$ and $CD8^+$ T-cells and total lymphocytes and normal hemoglobin concentrations compared with the control group (57). Supplementation with *Akkermansia muciniphila* increased the efficacy of anti-PD-1 immunotherapy in antibiotic-treated mice (40). In a mouse model of melanoma, supplementation with *Bifidobacteria* improved the response to anti-PD-L1 treatment and almost eliminated cancer growth (28,58). Certain probiotic strains that may be associated with the efficacy of ICI therapy have been tested in GF or SPF animal models of cancer (31). At present, the ability of probiotics to regulate the intestinal microbiota and thereby improve the efficacy of ICI therapies are being

Table II. Selected registered immunotherapy studies evaluating therapeutic roles of gut microbiota.

Identifier	Study title	Status	Phase	Condition or disease	Intervention/treatment
NCT03341143	Fecal Microbiota Transplant (FMT) in Melanoma Patients	Recruiting	Phase 2	Melanoma	Drug: FMT with Pembrolizumab
NCT04130763	Fecal Microbiota Transplant (FMT) Capsule for Improving the Efficacy of Anti- PD-1	Recruiting	Phase 1	Gastrointestinal cancer	Biological: FMT capsule
NCT04116775	Fecal Microbiota Transplant and Pembrolizumab for Men with Metastatic Castration-Resistant Prostate Cancer.	Recruiting	Phase 2	Prostate cancer; metastatic prostate cancer	Biological: FMT; Drug: Pembrolizumab; Drug: Enzalutamide
NCT03353402	Fecal Microbiota Transplantation (FMT) in Metastatic Melanoma Patients Who Failed Immunotherapy	Recruiting	Phase 1	Melanoma stage IV; unresectable stage III melanoma	Procedure: FMT
NCT03772899	Fecal Microbial Transplantation in Combination with Immunotherapy in Melanoma Patients (MIMic)	Recruiting	Phase 1	Melanoma	Drug: FMT

FMT, Fecal Microbial Transplantation.

investigated in clinical trials. One such trial is aiming to investigate the efficacy of the probiotic strain *Clostridium butyricum* CBM588 when combined with nivolumab and ipilimumab for the treatment of kidney cancer (trial no. NCT 03829111).

Prebiotics are defined as inactive food ingredients that selectively promote the growth and activity of one or several microorganisms in the colon, and are thus beneficial to the health of the host (59,60). Prebiotics mainly comprise dietary fiber, and the short-chain fatty acids produced by the metabolism of these ingredients may decrease intestinal pH and maintain the growth of beneficial bacteria, including lactic acid bacteria and bifidobacteria, in the gut (61). Resistant starch produced by prebiotics promotes the growth of strains that produce butyric acid, which has anticancer and anti-inflammatory activities (62). These effects may be the mechanism by which prebiotics regulate the outcomes of immunotherapy (58).

Symbiotics are combinations of prebiotics and specific probiotic bacteria (11) that have synergistic effects and may potentially improve the efficacy of immunotherapy. To date, the application of symbiotics in anticancer immunotherapy has been less thoroughly studied. However, these approaches combine the advantages of predominant probiotic bacteria and prebiotics and therefore may be promising.

In summary, probiotics, prebiotics and symbiotics may improve the outcomes of cancer immunotherapy, and they require further investigation.

Lifestyle. The lifestyle of the whole human organism, including exercise habits, dietary intake and sleep patterns, has significant effects on the gut microbiota, and the resulting changes may influence the efficacy of cancer immunotherapy. For example, it has been demonstrated in exercise oncology studies that exercise may regulate the cancer microenvironment and enhance the response to cancer immunotherapies (63). Clarke *et al* (64) studied the effects of exercise on the gut microbiota of athletes and found that highly active rugby players had significantly more diverse gut microbiota and lower levels of inflammatory and metabolic biomarkers, compared with the control group. It has been demonstrated in other studies that lactic acid may regulate the expression of PD-L1 in cancer cells (65), while the decrease in lactic acid concentrations in cancer may increase the number of tumor-infiltrating immune cells (66). Exercise may decrease lactic acid concentrations and may thus contribute toward the efficacy of cancer immunotherapy.

It was determined in dietary studies that the profiles of the gut microbiota of research subjects who consumed high-fat diets were significantly different to those of research subjects who consumed fiber-rich diets (67). The diet serves an important role in shaping the composition and activity of the complex gut microbiota (68) and may therefore contribute toward the efficacy of immunotherapy. Regarding sleep, a late bedtime may disrupt the balance of the gut microbiota and regulate the host's metabolism (69). Studies have identified a potentially close association between sleep quality and the gut microbiome composition (70). Furthermore, mouse experiments have confirmed that chronic sleep disruption alters the gut microbiota composition (71). In summary, lifestyle factors, including exercise, diet and sleep may regulate the gut microbiota and may impact the efficacy of cancer immunotherapy.

Traditional chinese medicine. A number of previous studies have demonstrated the effects of components of Traditional Chinese Medicine on the gut microbiota. For example, a combination of the Chinese herbal compound Gegen Qinlian decoction (GQD) with anti-PD-1 immunotherapy may be a novel strategy for the treatment of microsatellite stable (MSS) colorectal cancer (CRC). In a mouse xenograft tumor model, combined treatment with GQD and an anti-mouse PD-1 antibody significantly inhibited the growth of CT26 tumors.

An analysis of the gut microbiota in these mice revealed that the combination therapy led to the significant enrichment of *Bacteroides acidophilus* and members of the S24-7 family (72). Shaoyao Ruangan mixture was demonstrated to increase the abundance of *Bacteroides* spp. and effectively inhibit the progression of primary liver cancer (73).

In terms of small molecules, it has been demonstrated that curcumin may increase the abundance of bacteria, particularly *Lactobacillus* spp (74). Furthermore, ginsenosides may restore the structure of the intestinal mucosa and improve immunity in this tissue, thereby promoting the growth of beneficial bacteria and decreasing the growth of bacteria that results from cancer-associated cachexia (75). Berberine may increase the relative abundance of the thick-walled *Mycoplasma* spp., decrease the relative abundance of proteobacteria and decrease ileal inflammatory responses and intestinal mucosal damage (76).

In summary, Traditional Chinese Medicinal mixtures and small molecules may regulate the gut microbiota and thus affect the outcomes of immunotherapy. The mechanisms by which Traditional Chinese Medicines regulate the gut microbiota in this context remain unclear, its prospective applications are broad and require further investigation.

6. Conclusions

There is increasing evidence of an association between the gut microbiota and the outcomes of cancer immunotherapy. In addition, it has been demonstrated that the efficacy of cancer immunotherapy may be optimized by adjusting the gut microbiota. Broadly, the evidence has suggested that methods and tools should be developed to enable adjustment of the composition and concentration of intestinal microbiota and thereby improve the prognosis of cancer immunotherapy.

Numerous methods are available for regulating the gut microbiota, but their mechanisms of action are unclear, the clinical sample sizes are small and risk-assessment data are lacking. Therefore, these methods are not widely used, and further investigation is required to ensure their uptake.

The gut microbiota is a huge and complex system that is influenced by numerous factors, and is associated with host immunity. It has been reported that gut microbiota and host immunity are associated via diet, intestinal absorption and enterohepatic circulation (77,78). Therefore, it may be a promising research direction to determine the association between gut microbiota and immunotherapy. At the same time, the use of recently introduced scientific and technological methods, including next-generation sequencing, big-data analysis and artificial intelligence, will enable further investigation into the relationship between the gut microbiota and immunotherapy. In particular, the identification of specific gut microbes that yield clear benefits during immunotherapy and the determination of the composition and proportion of the gut microbiota will facilitate screening to determine individual patients' suitability for immunotherapy. The use of specific and effective methods to regulate the gut microbiota, and the careful and comprehensive observation of its role in immunotherapy regulation, are required to develop consensus-based guidelines for clinical application of this promising adjunctive cancer treatment.

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Authors' contributions

JW and XXW wrote and edited the manuscript. HRY and DJW contributed toward the conception and design of the study and critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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