Review Article

Efficacy and regulatory strategies of gut microbiota in immunotherapy: a narrative review

Yaodong Sang[1#], Kexin Zheng[2#], Yulong Zhao[1], Yuan Liu[2], Siqiang Zhu[1], Xiaozhou Xie[1], Liang Shang[1,2], Jin Liu[3], Leping Li[1,2]

[1]Department of Gastrointestinal Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China; [2]Department of Gastrointestinal Surgery, Shandong Provincial Hospital, Shandong University, Jinan, China; [3]Department of Gastroenterology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China

Contributions: (I) Conception and design: J Liu, L Li, L Shang; (II) Administrative support: Y Sang, K Zheng, Y Liu; (III) Provision of study materials or patients: K Zheng, Y Zhao; (IV) Collection and assembly of data: Y Sang, Y Zhao; (V) Data analysis and interpretation: X Xie, S Zhu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Jin Liu, MD. Department of Gastroenterology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324 Jingwu Road, Huaiyin District, Jinan 250000, China. Email: miracle713@163.com; Leping Li, MD. Department of Gastrointestinal Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324 Jingwu Road, Huaiyin District, Jinan 250000, China; Department of Gastrointestinal Surgery, Shandong Provincial Hospital, Shandong University, Jinan, China; Email: lileping@medmail.com.

Background and Objective: With advances in gut microbiome research, it has been recognized that the gut microbiome has an important and far-reaching impact on many human diseases, including cancer. Therefore, more and more researchers are focusing on the treatment of gut flora in tumors. In this article, we present a review of the mechanisms of gut microbes in tumor immunotherapy and related studies to provide reference for further research and insights into the clinical application of gut microbes.

Methods: Between April 25, 2023, and November 25, 2023, we searched for articles published only in English between 1984 and 2023 using the databases PubMed, American Medical Association and Elsevier ScienceDirect using the keywords "gut microbiology" and "tumor" or "immunotherapy".

Key Content and Findings: The gastrointestinal tract contains the largest number of microorganisms in the human body. Microorganisms are involved in regulating many physiological activities of the body. Studies have shown that gut microbes and their derivatives are involved in the occurrence and development of a variety of inflammations and tumors, and changes in their abundance and proportion affect the degree of cancer progression and sensitivity to immunotherapy. Gut microbiota-based drug research is ongoing, and some anti-tumor studies have entered the clinical trial stage.

Conclusions: The abundance and proportion of intestinal microorganisms influence the susceptibility of tumors to tumor immunotherapy. This article reviewed the effects and mechanisms of gut microbes on tumor immunotherapy to further explore the medical value of gut microbes in tumor immunotherapy.

Keywords: Gut microbiota; immunotherapy; fecal microbiota transplantation (FMT)

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Introduction

The gut microbial community, defined as the microbiome, consists of bacteria, fungi, viruses, archaea, and protists that play a vital role in maintaining human health and fighting disease. More than 100 trillion microorganisms reside in the gastrointestinal tract, which is more than that in any other part of the human body (1). 16S ribosomal RNA (rRNA) sequencing has shown that the normal intestinal

flora of the human gut is mainly composed of firmicutes and *Bacteroides*, while proteobacteria, actinomycetes, clostridiales, and verrucobacteria are less abundant (2). The diversity, abundance, and proportion of human intestinal flora remains stable in a healthy individual. However, the abundance and proportion of bacteria in different parts of the digestive tract differ. From the esophagus to the rectum, the number of bacteria in each gram of intestinal content gradually increases from 10 to 10^{12} CFU/mL (3). For example, the dominant bacterium in the distal esophagus and duodenum is *Streptococcus*, while in the colon and rectum, *Fusobacterium* predominates (4,5). Since the number of bacteria in the colorectum accounts for 70% of the total number of intestinal microorganisms, the intestinal flora usually discussed in the context of disease states refers to the colonic flora (6).

Intestinal microbes rooted in humans evolve a strong reciprocal symbiotic relationship with their hosts (7). Using this relationship, the host provides a habitat and nutrients for microorganisms (8) and coordinates integration and metabolic signals, microbial sensing information, and immune response pathways to ensure the normal operation of its own function. Intestinal microorganisms produce and regulate various metabolites (including bioactive compounds such as short-chain fatty acids), prevent infection by foreign pathogens, control pathological overgrowth, regulate mucosal barrier function, maintain mucosal immune homeostasis, ensure the stability of host-microbe symbiosis, regulate intestinal endocrine and neurological functions, and promote the normal function of the host immune response (9-13). With a biomass of 1.5 kg, the abundance and proportion of the gut microbiota is influenced not only by the interaction between the microbial community and the host but also by age, diet, medication, environment, sex, and ethnicity (14-17). Significant changes in any of the above factors will lead to intestinal dysbiosis.

Intestinal flora imbalance refers to the destruction of microbial ecosystems under the influence of environmental and host-related factors, resulting in changes in the proportion and function of the intestinal flora. Dysbiosis of the gut microbiota exerts detrimental effects on host health by altering the gut microbiota itself, influencing metabolic activity and/or changing the local abundance (18). The occurrence of a series of human diseases is also closely related to such changes. These include inflammatory bowel disease (19), ankylosing spondylitis (20), and metabolic abnormalities such as obesity and diabetes (21), as well as many types of cancer (22-25). This review focuses on the interaction between intestinal flora and tumor immunotherapy to provide new ideas for optimizing tumor immunotherapy. We present this article in accordance with the Narrative Review reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-316/rc).

Methods

A literature search was performed in the databases PubMed, American Medical Association, and Elsevier ScienceDirect using the keywords "gut microbiology" and "tumor" or "immunotherapy". In addition, secondary references cited in the articles retrieved by PubMed were also retrieved. *Table 1* summarizes the search methodology.

Gut microbiota, immunity, and cancer

The gastrointestinal tract is the immune organ with the largest contact area between the body and the external environment and is an important barrier against external pathogenic factors. Stem cells located at the base of the intestinal crypt can differentiate into intestinal epithelial cells (IECs) and intraepithelial lymphocytes (IELs) with special functions, which make up the mucosal layer. IECs and goblet cells produce antimicrobial peptides and mucins, respectively, which play important roles in pathogen control, lubrication, and the protection of the intestinal epithelium. Goblet cells also act as antigen-presenting cells (APCs) by supplying antigens to dendritic cells (DCs). This also promotes the development of regulatory T cells (Tregs). Below the mucosal layer, the lamina propria (LP) contains a large number of immune cells, such as APCs, DCs, and intestinal-associated lymphoid tissues, the latter consisting of Peyer's patches, LP lymphocytes, and IELs. These cells synergistically influence and regulate the body's local and systemic immune responses.

The immune recognition of microorganisms in host innate immunity is mainly based on two pattern recognition receptor (PRR) systems; that is, Toll-like receptors (TLRs) and intracellular nucleotide-binding oligomerization domain receptors, which can modulate immune cells to play a role in both innate immunity and adaptive immunity (26,27). PRRs are widely distributed in various immune cells in the gut, such as IECs, macrophages, and DCs (18). PRRs can recognize microbial-associated molecular patterns (MAMPs) and pathogen-associated molecular patterns (PAMPs) (28). Host commensal bacteria can reduce the

Table 1 Methodology of the search for the review

Items	Specification
Date of search	April 25, 2023 to November 25, 2023
Databases and other sources searched	PubMed, American Medical Association, Elsevier ScienceDirect
Timeframe	1984 to 2023
Search terms used	"gut microbiology" and "tumor" or "immunotherapy"
Inclusion criteria	Restricted to articles published in English
Selection process	Seven authors, Y.S., K.Z., Y.Z., X.X., S.Z., Y.L., and L.S., independently conducted literature searches in three databases: PubMed, American Medical Association, and Elsevier ScienceDirect. The three databases are independent of each other, ensuring the diversity and breadth of search results. After completing their respective searches, two authors, J.L. and L.L., participated in the review and discussion of the literature, and finally reached a consensus on the collected literature

migration of immune cells and present autoantigens to the surrounding lymphoid tissue through phagocytes to activate immune cells and exert immunoprotective effects. Phagocytes can stably express pro-interleukin-1 beta (IL-1β) to meet the rapid maturation of IL-1β in response to bacterial infection. This process acts through a myd-88-dependent mechanism (29,30). Pathogens stimulate immune cells to produce proinflammatory cytokines, thereby stimulating immune regulation (18). For example, during infections by organisms such as *Salmonella*, the proinflammatory factor IL-1β rapidly recruits neutrophils to maintain immune homeostasis, but when myd-88 is dysregulated, the abundance of bacteria increases (18).

Intestinal draining lymph nodes, found in the mesentery, are called mesenteric lymph nodes (mLNs). In mLNs, the microbiota can also stimulate adaptive immunity, and MAMPs and PAMPs can induce the maturation of a variety of APCs, including DCs (31,32). Mature DCs enter mLNs and promote the conversion of naive T cells into $CD4^+$ T cells and $CD8^+$ T cells (26,31). A proportion of DCs and activated immune cells can enter the immune circulation through mLNs and exert a broader immune effect (32). Not only can bacteria themselves trigger an immune response, but bacteria and their own components can also stimulate immunity. For example, *Bacteroides fragilis* polysaccharide A can be presented to T cells via DCs to stimulate IL-10 production, whereas $CD4^+$ T cells can also stimulate the regulation of the number and frequency of $Foxp3^+$ Treg differentiation, further stimulating IL-10 production (33-36) (*Figure 1*).

The current study found that microbes exert a carcinogenic effect in two ways. First, the microbiome directly generates toxic metabolites or carcinogenic products as cancer-converting agents (37). The intestinal flora can produce some carcinogenic metabolites, including sulfides, ammonia, and nitrosamines, by digesting protein substances. High-protein diets have been reported to increase the production of toxic metabolites and decrease the production of anti-cancer metabolites, thereby increasing the risk of cancer (38). These metabolites can cause oxygen radical formation and DNA mutations, which in turn can lead to the development of cancer (39). Second, microorganisms can also directly promote the occurrence of cancer by inducing carcinogenesis (37). Fecal transplants from patients with colorectal cancer promote carcinogenic effects in sterile and conventional mice. In a mouse model of ulcerative colitis cancer established by nitromethane (acute otitis media)/sodium dextran sulfate, the authors found that the microbiota of the metastasized tumor-carrying mice accelerated the development and progression of tumors (40). In addition, the structure and physiological state of microorganisms also exert carcinogenic effects, and biofilm-associated communities from colorectal cancer patients and healthy individuals are more likely to induce tumorigenesis than non-biofilm communities in mouse models (41). These findings all suggest that the impact of the microbiome on cancer treatment is not limited to one pathway, which has also sparked interest in studying the mechanisms by which gut microbes function in the gut. Below, we discuss some of the existing registered clinical studies (*Table 2*).

Intestinal microbiota and tumor immunotherapy

At present, there are five main types of immunotherapy:

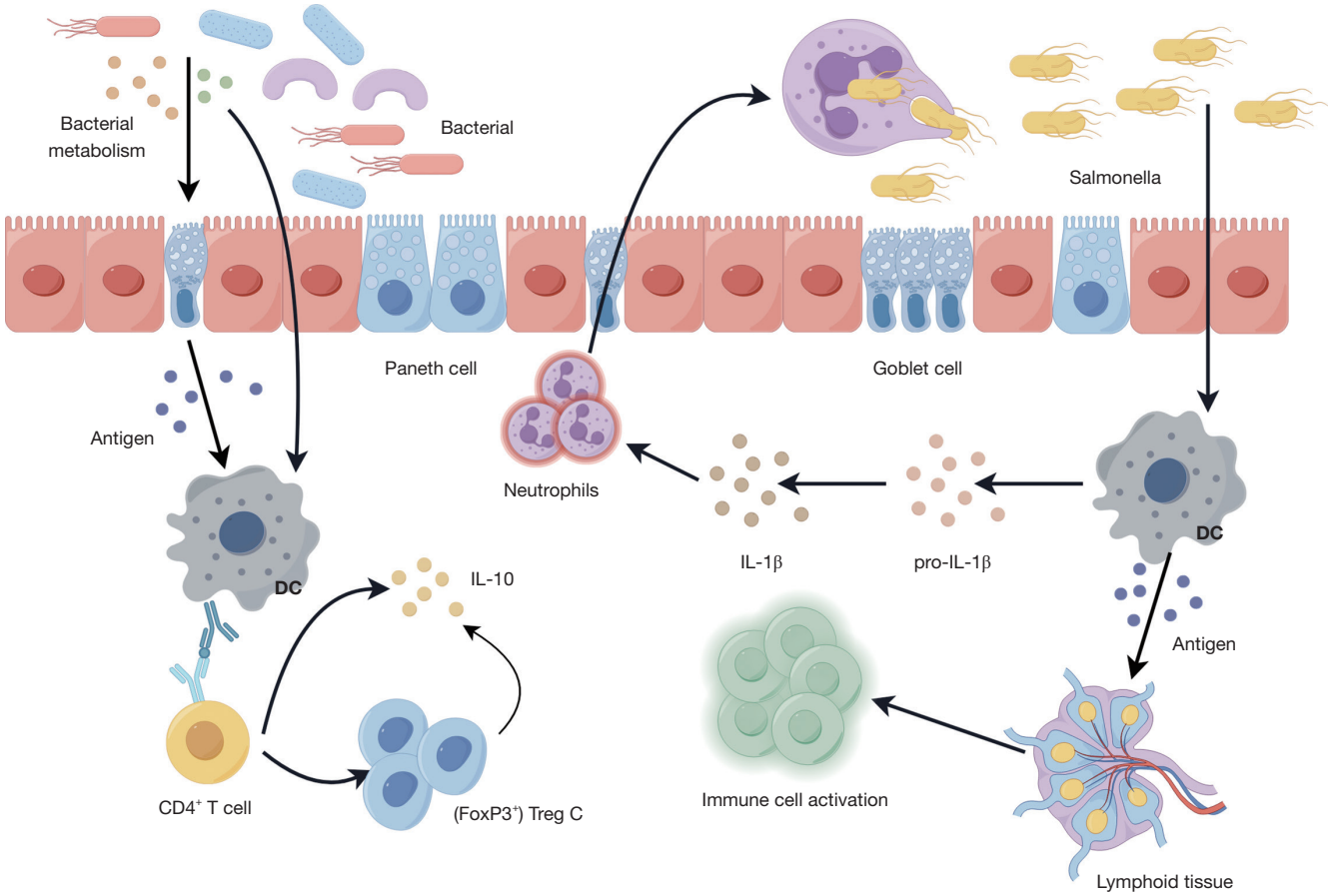

Figure 1 The bacteria stimulate goblet cells to deliver antigens to DCs, which in turn stimulate Foxp3$^+$ regulatory T cells and produce IL-10 via CD4 T cells. Bacteria can directly stimulate phagocytes to present antigens to lymphoid tissue, thus activating immune cells. For example, *Salmonella* directly stimulates phagocytic cells to produce IL-1β, increasing the maturity and number of IL-1β, thereby promoting neutrophil recruitment. *Figure 1* created by figdraw.com. DC, dendritic cell; IL, interleukin.

Table 2 Existing registered immunotherapy studies evaluating the prognosis and therapeutic effects of the microbiota

Research title	ClinicalTrials.gov identifier	Status	Target population	Study type	Sponsor
Fecal Microbiota Transplant (FMT) in Melanoma Patients	NCT03341143	Active, not recruiting	• Melanoma	• Phase 2	• Zarour, Hassane, MD • Merck Sharp & Dohme LLC
A Phase Ib Trial to Evaluate the Safety and Efficacy of FMT and Nivolumab in Subjects With Metastatic or Inoperable Melanoma, MSI-H, dMMR or NSCLC	NCT04521075	Unknown status	• Melanoma stage IV • Unresectable melanoma • NSCLC stage IV	• Phase 1 • Phase 2	• Ella Therapeutics Ltd.

Table 2 (*continued*)

Table 2 (*continued*)

Research title	ClinicalTrials.gov identifier	Status	Target population	Study type	Sponsor
Microbiota Transplant to Cancer Patients Who Have Failed Immunotherapy Using Faeces From Clinical Responders (MITRIC)	NCT05286294	Recruiting	• Melanoma stage IV • Head and neck squamous cell carcinoma • Cutaneous squamous cell carcinoma • MSI-high • Clear cell renal cell carcinoma • NSCLC	• Phase 2	• Oslo University Hospital
Fecal Microbial Transplantation Non-Small Cell Lung Cancer and Melanoma (FMT-LUMINATE)	NCT04951583	Recruiting	• NSCLC (metastatic) • Advanced melanoma	• Phase 2	• Centre hospitalier de l'Université de Montréal (CHUM)
Fecal Microbial Transplantation in Combination With Immunotherapy in Melanoma Patients (MIMic)	NCT03772899	Active, not recruiting	• Melanoma	• Phase 1	• Lawson Health Research Institute
Feasibility Study of Microbial Ecosystem Therapeutics (MET-4) to Evaluate Effects of Fecal Microbiome in Patients on Immunotherapy (MET4-IO)	NCT03686202	Active, not recruiting	• All solid tumors	• Phase 2 • Phase 3	• University Health Network, Toronto • NuBiyota
Fecal Microbiota Transplant and Re-introduction of Anti-PD-1 Therapy (Pembrolizumab or Nivolumab) for the Treatment of Metastatic Colorectal Cancer in Anti-PD-1 Non-responders	NCT04729322	Active, not recruiting	• Metastatic colorectal adenocarcinoma • Metastatic small intestinal adenocarcinoma • Stage IV colorectal cancer • Stage IV small intestinal adenocarcinoma	• Phase 2	• M.D. Anderson Cancer Center • National Cancer Institute (NCI)
CBM588, Nivolumab, and Ipilimumab in Treating Patients With Stage IV or Advanced Kidney Cancer	NCT03829111	Active, not recruiting	• Renal cell carcinoma	• Phase 1	• City of Hope Medical Center • National Cancer Institute (NCI)
Fecal Microbiota Transplantation to Improve Efficacy of Immune Checkpoint Inhibitors in Renal Cell Carcinoma (TACITO)	NCT04758507	Active, not recruiting	• Renal cell carcinoma	• Phase 1 • Phase 2	• Fondazione Policlinico Universitario Agostino Gemelli IRCCS
Fecal Microbiota Transplant and Pembrolizumab for Men With Metastatic Castration Resistant Prostate Cancer	NCT04116775	Recruiting	• Prostate cancer • Prostate cancer (metastatic)	• Phase 2	• Merck Sharp & Dohme LLC Prostate Cancer Foundation • Johns Hopkins University • Oregon Health and Science University

NSCLC, non-small cell lung cancer; MSI, microsatellite instability.

(I) molecular targeted therapy; (II) immune checkpoint inhibitor (ICI) therapy [programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) inhibitors]; (III) adoptive immune cell therapy (CAR-T, TIL, NK, and CIK/DC-CIK); (IV) cytokine therapy; and (V) tumor vaccines (e.g., Provenge and CIMAvax). The activation of CTLA-4, PD-1, or PD-L1 adversely affects the host, downregulating the body's immune response to tumors. Tumor cells in the tumor microenvironment (TME) overexpress these molecules to evade anti-tumor immune surveillance (42). The TME is often in a state of immunosuppression, avoiding detection by the immune system, and there is often almost no T cell infiltration in and around the tumor, which is commonly referred to as a "cold tumor". However, it should be noted that the overall immune status of such patients does not change significantly. Blocking these receptors or ligands can restore the patient's immune anti-tumor response. Immune checkpoint suppression is more tumor-specific than enhanced immunotherapy. Therefore, tumor immunotherapy research mainly focuses on tumor blockade therapy.

Currently, relatively well-established interventions include anti-$CD8^+$ T cell PD-1/PD-L1 antibodies and anti-CTLA-4 antibodies. These antibodies are called ICIs (42). The mechanisms of action differ; anti-CTLA-4 Ab acts primarily in the initial part of the immune response (43). PD-1 is expressed in activated T cells and lymphoid B cells. PD-1 phosphorylation occurs after binding to the B7 ligand PD-L1, inhibiting T cell proliferation and its associated immune response. Thus, targeting PD-1/PD-L1 inhibitors can increase T cell-mediated anti-tumor immune activity, ultimately achieving anti-tumor effects (44). To date, ICIs have been widely used in various cancer treatments and have shown good therapeutic efficacy in some patients. However, one study showed that the overall response rates are less than 30% for most tumor types (43). Fortunately, some studies (43-46) have shown that differences in gut microbes in cancer patients are related to the effectiveness of immunotherapy, which suggests a direction for further research. *Table 3* provides list of microorganisms that have been shown to play a role in immunotherapy. Gut microbes influence the sensitivity of tumors to various therapies, especially immunotherapy. Records of immunotherapy using microorganisms to treat cancer date back to the late 19th century, when 1,000 sarcoma patients were treated with a heat-killing mixture of *Streptococcus pyogenes* and *Serratia*, increasing their 5-year survival rate by 80% (55). Researchers have hypothesized that this mixture induces a sustained immune response and exerts anti-tumor effects (42). This effect on the human immune system makes it a key component of the TME, contributing to the therapeutic activity of CTLA-4- or PD-1/PD-L1-based cancer immunotherapies.

Regulation of anti-tumor immunity by the gut microbiota is a complex and diverse process involving multiple mechanisms. Firstly, modulation of antitumour immunity by gut flora through metabolites is one of the most dominant modalities. As metabolites are mostly small molecules, diffusion from the original location can be achieved to influence local and systemic anti-tumor immune responses, thus improving the effect of immunotherapy. Inosine belongs to the purine metabolites, which are normal metabolites in the human body and are often produced by is metabolism of *Bifidobacterium pseudolongum* and *Mucorophilus*. It has been found that inosine can up-regulate IL12Rβ2 and IFNγ transcription through a series of reactions, and promote the differentiation and accumulation of Th1 cells in TME, thus enhancing the efficacy of ICI (56). *In vitro* physiological concentrations of inosine enhance Th1 differentiation and effector function of initial T cells expressing A2AR (57). Interestingly, this enhanced differentiation is dependent on the presence of IFNg; when this cytokine is absent, inosine inhibits Th1 differentiation. Overtransfer of A2AR-deficient T cells into rag1-deficient mice reduced the immune checkpoint blockade (ICB) response to inosine-producing bacteria. Importantly, the ICB-promoting effects of these bacteria and of inosine supplementation were context-dependent: in the absence of treatment with CpG, a widely used anti-tumor adjuvant, inosine caused tumors to become larger and reduced anti-tumor immunity, rather than improving ICB responses (57). Effector T cells can also utilise inosine as an alternative substrate to support cell growth and function in the absence of glucose *in vitro*. In T cells, the ribose subunit of inosine has access to central metabolic pathways to provide ATP and precursors for biosynthesis in the glycolytic and pentose phosphate pathways, whereas a wide variety of different cancer cells show varying degrees of ability to utilise inosine as a carbon source. In addition, inosine supplementation enhanced the anti-tumor efficacy of ICB or overt T cell transfer in solid tumors defective in metabolising inosine (58). Undigested and absorbed carbohydrates or glycoproteins secreted by IECs can be digested by colonic anaerobes to produce short-chain fatty acids. Butyric acid

Table 3 Summary of characteristic microorganisms in microbiome immunotherapy studie

Bacteria	Model	Study drug	Sample	Methods	Main findings	Target population	Author/ year/ref.
Alistipes shahii	Mouse	IL-10/CpG, oligonucleotide, immunotherapy	Feces	• 16S rRNA sequencing	Positive correlation of *Alistipes shahii* with TNF production and immunotherapy response	Lymphoma Melanoma Colon carcinoma (subcutaneous)	Iida N, 2013 (47)
Bacteroides thetaiotaomicron	Mouse	CTLA-4 mAb	Feces	• Microbiota evaluation with high-throughput sequencing of 16S rRNA gene amplicons	Increased levels of *Bacteroides thetaiotaomicron* and *Bacteroides fragilis* in ICI responders; improved response to CTLA-4 blockade with FMT from patients with increased fecal *Bacteroides* spp. levels	Melanoma	Vétizou M, 2015 (48)
Bacteroides fragilis	Human			• FMT			
Bifidobacterium spp.	Mouse	PD-L1 mAb	Feces	• 16S rRNA sequencing • FMT	The effect of oral *Bifidobacterium* spp. blocking PD-L1 on tumor control was similar to that of ICI responders.	Melanoma	Sivan A, 2015 (49)
Bifidobacterium longum, *Collinsella aerofaciens*, *Enterococcus faecium*	Mouse Human	PD-1 mAb	Feces	• 16S rRNA sequencing • Metagenomic shotgun sequencing • qPCR • FMT	Increased *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* in the feces of ICI responders	Melanoma	Matson V, 2018 (50)
Ruminococcaceae, *Bacteroidales*	Mouse Human	PD-1 mAb	Feces Buccal	• 16S rRNA sequencing • Metagenomic whole genome shotgun sequencing • FMT	Higher alpha diversity and increased *Ruminococcaceae* levels in the feces of ICI responders; higher buccal and fecal levels of *Bacteroidales* in ICI non-responders; restoration of the efficacy of PD-1 blockade in antibiotic pretreated mice after FMT from ICI responders	Melanoma	Gopalakrishnan V, 2018 (51)

Table 3 (*continued*)

Table 3 (*continued*)

Bacteria	Model	Study drug	Sample	Methods	Main findings	Target population	Author/ year/ref.
Enterococcaceae, *Enterococcus*, *Streptococcus australis*	Human	PD-1 mAb	Feces	• Microbiota evaluation 16S rRNA gene and metagenomics sequencing • FMT	*Enterococcaceae*, *Enterococcus*, and *Streptococcus australis* increased in patients who responded better to PD-1 treatment	Melanoma	Baruch EN, 2021 (52)
Bacteroides stercoris, *Parabacteroides distasonis*, *Fournierella massiliensis*	Human	CTLA-4 mAb, PD-1 mAb	Feces	• 16S rRNA gene sequencing • Whole metagenomic shotgun sequencing	The increase of *Bacteroides stercoris*, *Parabacteroides distasonis*, and *Fournierella massiliensis* was positively correlated with the therapeutic effect of CICB	Melanoma	Andrews MC, 2021 (53)
Ruminococcus SGB15229 and *SGB1505*, *Eubacterium ramuleus*, *Eubacterium*	Human	PD-1 mAb	Feces	• Microbiota evaluation 16S rRNA gene and metagenomics sequencing • FMT	Increased *Eubacterium ramuleus*, *Eubacterium*, *Ruminococcus SGB15229*, and SGB1505 in the feces of ICI responders	Melanoma	Routy B, 2023 (54)

IL-10, interleukin 10; rRNA, ribosomal RNA; TNF, tumor necrosis factor; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; FMT, fecal microbiota transplantation; ICI, immune checkpoint inhibitor; PD-L1, programmed cell death-ligand 1; PD-1, programmed death 1; qPCR, quantitative real-time polymerase chain reaction; CICB, combined immune checkpoint blockade.

can increase the expression of ID2 in $CD8^+$ T cells and enhance the anti-tumor immune response of $CD8^+$ T cells, thus improving the effectiveness of anti-tumor therapy (59). Inhibition of histone deacetylase (HDAC) class I enzymes by butyric and valeric acids reprograms $CD8^+$ T cells, resulting in increased production of pro-inflammatory and cytotoxic molecules, which in turn enhances the anti-tumor activity of immune cells (60).

Secondly, the gut microbiota can also act as messengers, transmitting signals from the gut and/or gut-associated lymphoid tissue (GALT) to distant tumor sites. The presence of these signals can influence the migration and function of immune cells, enabling them to perform immunostimulatory or suppressive functions at the tumor site. Intestinal flora organisms can initiate tumor-infiltrating myeloid cells via TLR4, and tumor necrosis factor produced by such cells upon stimulation rapidly induces hemorrhagic necrosis, which ultimately exerts an anti-tumor effect via $CD8^+$ T cells, resulting in delayed tumor growth and prolonged survival (47,61). Paulos *et al.*'s study found that lipopolysaccharides (LPS) released after intestinal radiation damage activated the natural immune response stimulated by the TLR4 pathway and promoted $CD8^+$ T cell proliferation, resulting in a significant increase in the efficacy of anti-tumor $CD8^+$ T cells against overt metastasis (62).

In addition, certain strains of the gut microbiota can promote the proliferation and activity of immune cells and enhance the body's ability to attack tumor cells. It was found that mucin Ackermannia could induce the activation of M1-type macrophages in the tumor immune microenvironment (63); *Pseudomonas fragilis* induces macrophage polarisation towards the M1 phenotype (64).

However, it should be noted that the relationship between gut flora and tumours is complex and not all gut microbes have a positive effect on anti-tumour immunity. Some metabolites may have a high carcinogenic risk, and overproduction may increase the risk of tumour development. Therefore, further in-depth studies are needed to explore the mechanisms involved.

Two articles published in *Science* in 2015 on CTLA-4 and PD-L1 antibody studies paved the way for microbiome studies on ICIs (48,49). Vétizou *et al.* investigated the

relative therapeutic effect of CTLA-4-specific antibodies on MCA205 sarcoma in mice under specific non-pathogenic (SPF) and sterile (GF; germ-free) conditions and found that tumor growth was controlled in the SPF group and not in the GF group (48). At the same time, the anti-tumor effect of CTLA-4-specific antibodies was also attenuated in mouse models treated with broad-spectrum antibiotics alone or imipenem, which also supports the involvement of the gut microbiota in the anti-cancer effect exerted by the CTLA-4 blockade (43). To explore whether the commensal microbiota affects the immune response of the tumor and thus the therapeutic activity of the immunotherapy intervention, the researchers conducted experimental exploration. By comparing the growth of subcutaneous melanoma in mice from two different sources (JAX and TAC), it was found that the tumors of the TAC mice grew faster. After feeding both mice together, it was found that the TAC mice gradually exhibited the same phenotype as the JAX mice. The researchers conjectured that the gut of the JAX mice may be inhabited by microorganisms with anti-tumor immunity. To examine the effectiveness of microbial transplantation, the feces of the two mice were transplanted into each other, and it was found that the tumor growth rate of the JAX mice was significantly slower, and the effect of combined treatment with anti PD-L1 mAb (aPD-L1mAb) was more obvious. The JAX mice also outperformed the TAC mice when treated with aPD-L1 alone, suggesting that commensal microorganisms influence spontaneous anti-tumor immunity and response to aPD-L1mAb immunotherapy. The researchers then analyzed two types of mouse feces using 16S sequencing technology and found that *Bifidobacterium* (false discovery rate: 0.0019) was positively correlated with anti-tumor T cell responses. After treating mice with *Bifidobacterium* alone, the tumors improved significantly. This finding also provides further evidence that commensal bacteria are capable of influencing the immune response of tumors (44).

Two important articles illustrate the same point from different perspectives: the efficacy of immunotherapy requires the involvement of gut microbes for modulation, and this microbiome is not confined to one type. A systematic review analyzed the effects of gut microbiota on ICIs in a variety of solid tumors (45). The results showed that patients with microbiota rich in Verrucobacterium and Firmicutes generally showed higher sensitivity to ICIs, while patients rich in Proteus showed disappointing results. Similarly, Sivan *et al.* studied the efficacy of ICIs in the treatment of MC38 tumor models and found that *Bifidobacterium pseudocolonica* and *Lactobacillus johnsonii* significantly improved the ICI efficacy against PD-L1 and anti-CTLA-4 (49). Additionally, in a later study, Vétizou, who was aware that tumors in antibiotic-treated or sterile (GF) mice do not respond significantly to the CTLA-4 blockade, showed that when colonized by two *Bacteroides* species and one *Burkholderia* (Proteus) species, the anti-cancer response of CTLA-4Ab was restored in mice transplanted with colon and melanoma tumors (48).

Fusobacterium nucleatum (*F. nucleatum*) has also been shown to enhance the therapeutic effect of PD-L1 antibodies in colon cancer (47). Using 16S rRNA sequencing and shotgun sequencing to detect baseline stool samples from patients with metastatic melanoma receiving anti-PD-1 antibody therapy, Bifidobacterium was found to be overrepresented in responding patients (50). Gopalakrishnan *et al.* examined the oral and gastrointestinal microbiota of 112 patients receiving anti-PD-1 therapy and found a higher diversity of α in the fecal microbiota of responders and an increased abundance of the *Clostridium* and Rumen families. $CD8^+$ T cell infiltration is positively correlated with faces, rumen cocci, and clostridial abundance (51). The enrichment of *Bacteroides* in the oral and fecal microbiota of non-responders is also an important finding (51). These results also provide further evidence in support of previous conclusions.

The discovery of these "good bacteria" may facilitate the development of immune-enhancing adjuvants and the development of future immunotherapy interventions. The oral administration of probiotics containing bifidobacteria in mice with poor intestinal flora enhances the anti-tumor efficacy of PD-L1 (49), This effect is primarily due to enhanced DC maturation, which enhances tumor-specific $CD8^+$ T cell activity (49). Gao *et al.* found that a combination treatment of *F. nucleatum* and PD-L1 blockers significantly reduced tumor growth (as measured by tumor volume) and tumor weight compared to the *F. nucleatum* treatment alone (65). They also found that supplementation with *F. nucleatum* increased the proportion of CD8 TILs in mice with anti-PD-L1 monoclonal antibody, indicating that *F. nucleatum* may enhance the therapeutic effect of anti-PD-L1 monoclonal antibody by increasing CD8 TILs (65). bifidobacteria supplementation in colorectal cancer has also been shown to improve the therapeutic efficacy of PD-1 inhibitors (66). A phase I clinical trial conducted by Routy *et al.* evaluated the safety and efficacy of healthy donor fecal microbiota transplantation (FMT) in combination with the PD-1 inhibitor nabuliumab or pembrolizumab in 20

previously untreated patients with advanced melanoma, resulting in an objective response rate (ORR) of 65% (13 of 20). Four cases (20%) reported a complete response (CR) (54). These results are encouraging for subsequent studies and should instill confidence in researchers (67).

Based on the above we have summarized the close and complex relationship that exists between the gut microbiome, the cancer immune response and immunotherapy. The cancer immune response is the process by which the host immune system responds to cancer cells. This process consists of two phases: the infectious immune response and the adaptive immune response. In the infectious immune response, the immune system rapidly recognizes and attacks a number of easily identifiable microorganisms. The adaptive immune response, on the other hand, recognizes more complex foreign objects, such as cancer cells. T cells and B cells play a key role in this process by recognizing and attacking antigens that have been altered on the surface of the cancer cells, thus producing highly specific antibodies to recognize and attack the cancer cells. Immunotherapy, on the other hand, is a treatment that attacks cancer by activating or enhancing the patient's own immune response. This approach can greatly enhance the immune system's ability to attack and increase the success rate of curing cancer. However, the efficacy of immunotherapy is largely influenced by the patient's gut microbiome. A study found that the combination of specific bacterial strains in the gut microbiome is significantly associated with a patient's response to treatment (68). Patients who respond well to immunotherapy have a gut microbiome rich in certain beneficial bacteria and relatively low in certain harmful bacteria. Thus, the relationship between the gut microbiome, cancer immune response, and immunotherapy is intertwined and mutually reinforcing. The gut microbiome influences the cancer immune response by modulating the state of the host immune system, which in turn affects the efficacy of immunotherapy. At the same time, the success of immunotherapy in turn affects the composition and function of the gut microbiome.

In the era of immunotherapy, ICI has led to a revolutionary breakthrough in the treatment of advanced tumors, changed the treatment pattern of patients with advanced malignant tumors, effectively prolonged the survival of patients with advanced tumors, and continues to benefit more and more cancer patients. As with the adoption of any new therapy, the increased use of ICI therapies has resulted in an increase in unique treatment-specific toxicities; that is, immune-related adverse events (irAEs). These irAEs are caused by the unintended effects of ICI-mediated immune system activation and can occur in any organ system, limiting the benefits of the clinical drugs and even endangering the lives of patients in severe cases. A 2016 review reported that the overall incidence of serious or life-threatening irAEs (grade ≥3) was 20% to 30% in patients treated with ipilimumab, 10% to 15% in patients receiving anti-PD-1 agents, and 55% in patients receiving anti-CTLA-4/PD-1 combination therapy (69). Such a high incidence of irAEs has clouded the use of ICIs. Therefore, the researchers were eager to discover conditions that might ameliorate the high incidence of irae by studying gut microbes.

A growing body of research suggests that the gut microbiome may influence the onset and progression of irAEs. In previous melanoma studies, researchers found that the abundance and proportion of specific microbes (70) and the metabolic derivatives of certain microbes (71) were associated with the occurrence and/or severity of pathogenesis of irAEs. In a cohort study of ICIs for the treatment of thoracic tumors, researchers found significant differences in the composition of the microbiota between patients who developed and those who did not develop irAEs, such that those who developed irAEs had a higher abundance of *Erysipelatoclostridium* and a lower abundance of *Bifidobacterium*, *Faecalibacterium*, and *Agathobacter* at the genus level than those who did not develop irAEs. The researchers also found no significant difference in the alpha diversity between the two types of patients, and thus hypothesized that the occurrence of irAEs depends more on the presence or absence of certain organisms in the microbiota than on the abundance of species (72). *Bifidobacterium*, a common probiotic, was present in greater numbers in patients who did not develop irAEs, and as a common probiotic, *Bifidobacterium* prevents immune-associated colitis by stimulating the modulation of Tregs via IL-10 and lowering levels of the inflammatory factor IL-6 (53). A study of patients with pairs of tumor types found that the microbial biomarkers *Acidaminococcus* and *Turicibacter* were associated with ICI efficacy and irAEs (73). With the exploration of intestinal microbes, a strong correlation between the occurrence of irAEs and intestinal microbes has also been observed.

The effects of the gut microbiome on immunotherapy are well advanced in animal models; however, the progression of microbiome regulation in cancer immunotherapy is a very slow process. It is difficult to translate the discovery of the gut microbiota for immunotherapy into human

studies because the anatomy of the gastrointestinal tract and intestinal wall of humans and mice differ significantly, and most (85%) of the microbes colonizing the gut of mice are not found in humans. Thus, researchers need to continue to explore these areas of research.

Gut microbiota influences immunotherapy

Based on the above description of the relationship between gut flora and tumor immunotherapy, we have summarized how gut flora affects the efficacy of immunotherapy.

Immune regulation

The gut microbiota promotes and regulates natural and adaptive immunity and is essential for the development and function of the immune system. Some gut bacteria stimulate the activation and proliferation of immune cells and enhance their ability to recognize and attack cancer cells. For example, ATP molecules produced by bacteria resident in the gut activate immune cells in a network of small lymph node-like structures in the gut, which subsequently produce the host factor colony-stimulating factor 2 (CSF2) and stimulate monocytes in the structures to become response-ready macrophages (74). A team of researchers at the University of Calgary's Cumming School of Medicine has found that *Bifidobacterium pseudomallei*, *Lactobacillus johnsonii*, and *Serratia marcescens* can enhance the immune system's ability to recognize and "wipe out" cancer cells (56). In addition, *Lactobacillus royale* can also produce a compound called indole-3-aldehyde (I3A), which stimulates "killer" T cells in tumors and improves the effectiveness of immunotherapy (75). Therefore, the balance or lack thereof of the gut microbiota directly affects the efficacy of immunotherapy.

Metabolic effect

The gut microbiota can metabolize drugs and other compounds, thereby affecting their concentration and activity in the body. Some gut bacteria are able to metabolize drugs used in immunotherapy, making them more readily absorbed or more efficacious. Zimmermann *et al.* (76) conducted the first systematic study of microbial-drug interactions and found that at least 2/3 of the 271 selected clinical drugs could be metabolized by one or more strains, and experimentally validated 30 microbially-encoded enzymes with drug-metabolizing capacity that were able to convert 20 drugs into 59 candidate metabolites. This study complements previous studies on microbial-drug interactions with extensive experiments and analyses, deepens our understanding of microbiome mechanisms of drug metabolism, and provides a basis for rationally modulating the individual microbiome to alter microbiome-host interactions for optimal drug efficacy. It is believed that this study is an important guide for the study of gut microbes on the metabolism of immunologic drugs.

Competition suppression

Beneficial bacteria in the gut microbiota can compete with harmful bacteria for nutrients and living space, thus inhibiting the growth and reproduction of harmful bacteria. In cancer immunotherapy, some beneficial bacteria can reduce the number of harmful bacteria through a competitive inhibition mechanism, reducing the risk of cancer recurrence. In a study (77) exploring the relationship between gut microbes and gastric cancer treatment, researchers found that patients with higher relative abundance of lactobacilli in the gut tended to have higher microbiome diversity and that such patients responded significantly better to anti-PD-1/PD-L1 immunotherapy. In addition, lactobacilli can use lactic and acetic acid produced by dietary fiber to influence intestinal pH changes. They help maintain intestinal pH balance and promote the growth of beneficial and harmless intestinal bacteria, while discouraging the invasion and stay of harmful and opportunistic pathogenic bacteria (78).

Inflammatory response

The gut microbiota can influence the integrity of the intestinal mucosal barrier and modulate the intestinal inflammatory response. In cancer immunotherapy, excessive inflammatory response may lead to tissue damage and immunosuppression, whereas a balanced gut microbiota may reduce the inflammatory response and improve the efficacy of immunotherapy.

However, it is important to draw attention to the fact that the influence of the gut microbiota on cancer immunotherapy is complex and diverse, and the specific mechanism of action may vary depending on factors such as individual differences, cancer type and treatment regimen. Therefore, when developing a personalized cancer immunotherapy regimen, it is necessary to consider the state of the patient's gut microbiota and take appropriate

measures to regulate the balance of the gut microbiota in order to improve efficacy and reduce side effects.

Measures to regulate intestinal flora

The role that gut flora plays in cancer is not completely clear at this stage, after all, there are several clinical trials underway, so it is difficult to relatively systematically sort out the mechanisms and effective treatment modalities of gut flora in cancer immunotherapy. Fortunately, the studies (79-81) using gut flora in improving gastrointestinal disorders and modulating immune function are relatively well defined, so it is feasible to draw on them to extrapolate the role of gut flora for tumor immunotherapy. As mentioned earlier, intestinal flora can regulate the immune function of gastrointestinal tract, stimulate the development and differentiation of intestinal immune cells, enhance the intestinal mucosal barrier function, and prevent harmful substances and pathogens from invading the body. The emergence of common gastrointestinal diseases such as ulcerative colitis and Crohn's disease is strongly associated with intestinal flora, and the improvement of such diseases can be carried out using intestinal microorganisms. A prospective study showed that the group with the highest intake of dietary fiber (24.3 g/day) had a reduced risk of Crohn's disease of about 40%. This protective effect is mainly due to the fact that gut microbes can metabolize fiber into short-chain fatty acids, which activate G-protein-coupled receptors and activate Tregs, thus enhancing the immune tolerance of the gut mucosa (82). A previous article suggested that a compound produced by bacteria called 12,13-diHOME reduces the number and activity of Treg, leading to immune dysfunction in early childhood, making them more susceptible to allergies and asthma later in life (83). This idea was developed to help prevent the development and treatment of asthma in children.

The study of intestinal microorganisms functioning on top of other diseases provides help for subsequent guided research on the role of intestinal flora in tumors, as well as some regulatory measures of intestinal flora, which we expect to be able to provide suggestions for the prevention and treatment of the disease through the exploration of the regulatory measures of intestinal flora. Understanding the relationship between intestinal flora and immunotherapy, anti-tumor immunity, and the biological mechanism of immunotherapy response are crucial for the rational regulation of microbial activity to improve the efficacy of ICI therapy (84,85). The interventions affecting the therapeutic effect of ICIs are described in *Figure 2*.

FMT

FMT refers to the transplantation of beneficial functional bacteria from the feces of a healthy individual into a patient's intestine to achieve the reconstruction of intestinal flora to treat internal and extraintestinal diseases. This is the most direct way of changing the diversity of the intestinal flora. In the middle of the 20th century, in the United States, Dr. Esman successfully treated four patients with severe membranous enteritis with fecal enemas derived from healthy individuals, beginning a new chapter in the application of human feces in modern Western medicine. At present, FMT has achieved significant results in the treatment of *Clostridium difficile* infection in the clinical stage, and many clinical studies support its application (86-88). Released in 2023, the first fecal microbial therapy pharmaceutical represents a milestone in the prevention of recurrent *Clostridium difficile* infection after antibiotic treatment, and it opens the door to microbial therapy, which can provide lessons for other related therapies (89). Fortunately, FMT also plays an ideal role in treating diseases other than *Clostridium difficile* infection. The underlying mechanism has not yet been clearly studied, but we believe it may be related to the age, sex, region, genetics, and lifestyle of the patient.

As mentioned above, FMT has been tested in the preclinical phase, and some studies have shown the potential role of FMT in patients treated with ICIs. Wang *et al.* (90) reported on the first patient with refractory ICI-associated colitis and two patients with different solid tumors, all of whom were successfully treated with FMT from healthy donors. Several clinical trials are currently underway to test the safety and efficacy of FMT. A phase 1 clinical trial published in *Science* in 2021 evaluated the safety and feasibility of the reinduction of FMT and anti-PD-1 immunotherapy in patients with refractory metastatic melanoma. Two FMT donors in the trial both previously had metastatic melanoma and had received anti-PD-1 monotherapy and achieved a CR for at least one year. For the FMT recipients, the presence of disease metastasis was required. Prior to the start of the experiment, antibiotics were used to clear the recipients' original intestinal flora, and FMT was then administered by colonoscopy and oral administration to reinduce anti-PD-1 therapy. The

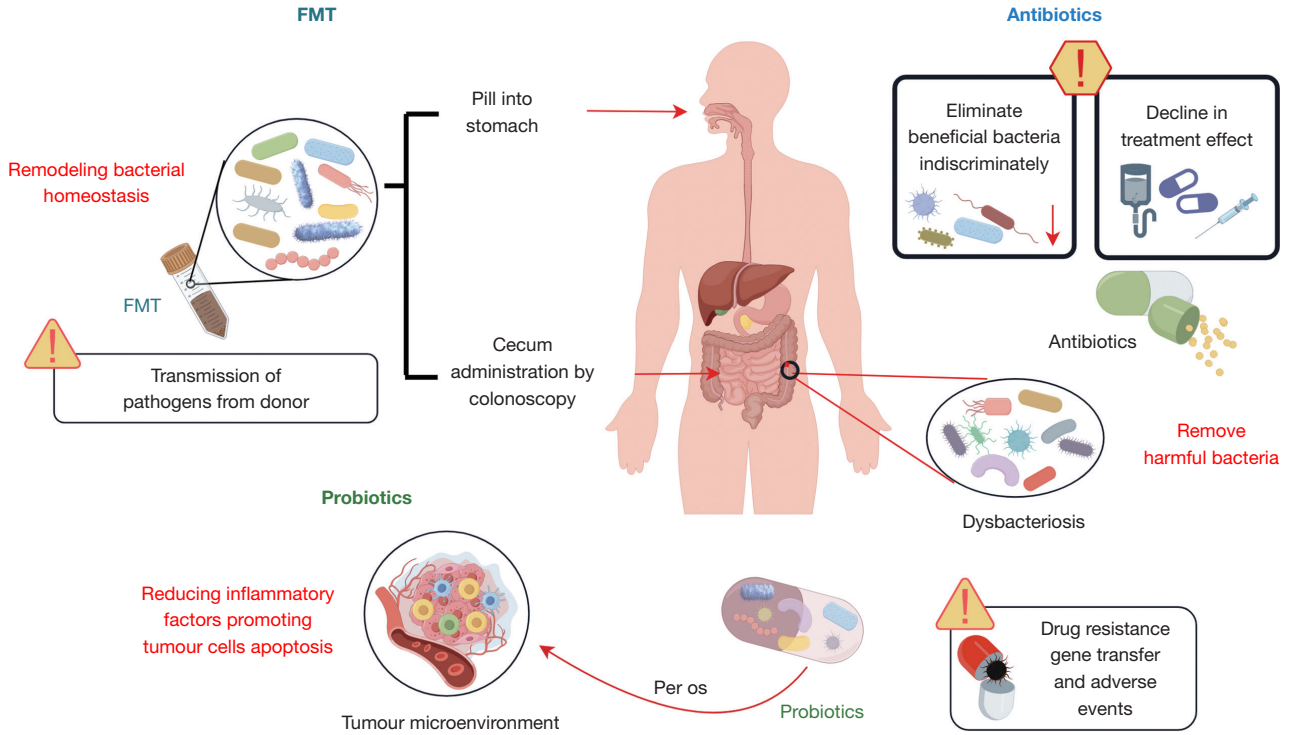

Figure 2 Fecal microbiota transplantation works by oral or colonoscopy transfer into the human body, including remodeling the bacterial abundance, but carries the risk of host antigen transplantation. Antibiotics kill bacteria indiscriminately; however, antibiotics also lead to a decrease in the effectiveness of immunotherapy. Probiotics change the flora abundance and proportion at the bacterial level; however, there is a risk of creating drug-resistant superbugs. *Figure 2* created by figdraw.com. FMT, fecal microbiota transplantation.

experiment lasted 90 days, with anti-PD-1 and oral fecal capsule infusions every 14 days to maintain FMT. During the trial, each of the 10 recipients received FMT from one of the two donors. Five recipients in the trial experienced a mild irAE (joint pain) when receiving the first anti-PD-1 treatment, but none of the recipients experienced moderate to severe irAEs throughout the trial. At the end of the trial, three recipients had an ORR to the treatment, one of whom achieved a CR, and two of whom achieved a partial response (PR). All of the recipients had a progression-free survival (PFS) of more than six months, which is an encouraging result (52). These results suggest that CR donor FMT and anti-PD-1 reinduction therapy are safe and feasible in patients with refractory metastatic melanoma. In some patients, this treatment increases immune activity in the tumor, which translates into an objective clinical response. However, it should be noted that the number of patients who received the experimental treatment was too small, and the trials conducted were not randomized to prove that FMT was an independent objective factor affecting anti-PD-1 therapy. Therefore, more controlled studies need to be conducted to answer this question. We also need to be cautious about the safety of FMT as a treatment.

Due to the complexity of the intestinal microbes and individual differences, the FMT mechanism is still unclear, and no universal "super stool" has been found. The transplantation of intestinal microbes may lead to complications, such as the spread of infection (91) and may increase the risk of autoimmune diseases (92). FMT is a donor-specific drug therapy, and its effects on the pathological conditions of different recipients are heterogeneous and cannot be uniformly evaluated. The limitations of widespread FMT use have inspired researchers to explore new treatments that can replace FMT. An article published in 2023 pioneered microbial ecosystem therapeutic 4 (MET4) (67), which balances FMT ecology and functional complexity, with practical application advantages. Composed of 30 different functional bacterial populations, including those associated with the immunotherapy response in previously published studies,

MET4 was cultured *in vitro*, each strain with specific genotypic and phenotypic characteristics. The study achieved major safety and tolerability endpoints and was the first study to explore alternative FMT microbiome combination therapies in patients receiving ICIs for advanced solid tumors. The results suggested that this combination therapy should be further explored in patients receiving ICI treatments.

Antibiotics

Antibiotics can be used to remove harmful bacteria and inhibit their growth, so they can alter the abundance and proportion of the microbiome and play a role in tumor development and tumor responses to treatment. However, due to the lack of targeted action of antibiotics against bacteria, this may also pose certain risks, including microbial imbalances and the development of emerging diseases. Several studies of different tumor types have shown that the efficacy of ICIs is inversely correlated with antibiotic use; that is, antibiotic use reduces the efficacy of ICIs in tumor treatments (93,94). The aforementioned study by Routy *et al.* also showed that antibiotic use affects the effectiveness of immunotherapy and thus patient outcomes, such that the subjects who used antibiotics 60 days before immunotherapy or within one month of immunotherapy initiation had shorter overall survival (OS) and PFS than nonusers (95). To investigate the effects of antibiotic use greater than 14 days and less than six weeks after ICI treatment on patient outcomes, a retrospective analysis was conducted of 291 patients with advanced cancer treated with ICIs, of whom 179 had melanomas, 64 had non-small cell lung cancers (NSCLCs), and 48 had renal cell carcinomas. In the study, 92 patients (32%) were treated with antibiotics. Patients who did not receive antibiotic therapy had the longest median PFS (6.3 months) and the longest median OS (21.7 months). After controlling for other clinically relevant factors, patients receiving a single course of antibiotics had a shorter median OS, and those who received multiple courses or long-term antibiotic therapy had the worst overall outcomes (94).

Broad-spectrum antibiotic mixtures impair the effectiveness of immunotherapy. A meta-analysis by Lurienne *et al.* found that the use of antibiotics before or during ICI treatment reduced the OS of NSCLC patients by more than six months (96). This may be the reason for the discovery of significantly reduced activation of spleen $CD4^+$ T cells and tumor-infiltrating lymphocytes in ATB-pretreated or GF mice. Most current studies have reported that antibiotics have negative effects on immunotherapy outcomes; however, some studies have suggested the opposite. One retrospective study of 74 patients with advanced NSCLC who were treated with Opdivo found that there was no significant difference in the ORRs and PFS between the antibiotic and non-antibiotic users (97). Other researchers have shown that antibiotic use has no effect on the major microbial composition that affects efficacy (98). This phenomenon differs from the findings of many previous studies and deserves further exploration. Most current data on the effects of antibiotics on the microbiome and immunotherapy are based on indirect effects analyses and retrospective studies, and it is clear that more prospective studies need to be conducted to explain this mechanism.

Probiotics

Probiotics are live microorganisms that benefit the host by colonizing the human body and altering the composition of the flora in a certain part of the host. By regulating the immune function of the host mucosa and system or the intestinal flora balance, probiotics engage in antipathogenic activity, regulate the immune system, reduce intestinal inflammation, and prevent cancer. Probiotics can be combined with cancer mutagens for biotransformation to achieve detoxification, which mainly depends on the peptidoglycans, polysaccharides, and glycoproteins on the probiotic surface. Probiotics can also downregulate the degree of inflammation, reduce the production of carcinogenic metabolites, and prevent cancer (99). A study by the investigators showed that the incidence of tumors in mouse models after treatment with *Clostridium butyricum* and 1,2-dihydrohydrochloric acid was reduced due to a decrease in the number of Th2 and Th17 cells, which in turn inhibited $CD4^+$ and $CD8^+$ T lymphocytes, blocking the cell cycle, reducing the secretion of inflammatory factors, and promoting the apoptosis of tumor cells (100). Another study found that cyclooxygenase-2 promotes tumor angiogenesis, while probiotics inhibit carcinogenesis by reducing the expression of cyclooxygenase-2 (101). A 2018 study showed decreased activity of the proteins glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and the gene catalase involved in apoptosis in rats treated with 1,2-dimethylhydrazine (DMH). However, after supplementation with *Lactobacillus plantarum* (AdF10) and

Lactobacillus rhamnosus GG (LGG), the activity of these enzymes increased again in the DMH-treated rats (102).

Probiotics may provide protection against oxidative stress and apoptosis-related protein dysregulation during experimentally induced colon cancer development. Sivan *et al.* found that probiotics enhance the anti-tumor effect of anti-PD-L1 drugs. bifidobacteria-treated mice show significantly improved tumor control, accompanied by the strong induction of peripheral tumor-specific T cells and increased accumulation of antigen-specific $CD8^+$ T cells in tumors (49). Researchers at the University of Pittsburgh (75) added commercially available probiotics to the diets of germ-free mice with melanoma, including *Lactobacillus reuteri*, *Bifidobacterium longum*, *Lactobacillus johnsonii*, and *Escherichia coli*, and found that after long-term feeding, the bacteria that entered the gut through the mouth migrated to the extrenteric tumors, and that any probiotic slowed tumor growth compared to the control mice, but *Lactobacillus reuteri* was particularly effective. Further research showed that there were more CD8 T cells in the experimental group, and *Lactobacillus reuteri* specifically activated the AhR receptor of $CD8^+$ T cells by secreting indole-3-formaldehyde, activated the AhR signaling pathway, promoted the phosphorylation of the transcription factor CREB, promoted the production of interferon gamma, and killed cancer cells. The study was the first to demonstrate that oral probiotics directly affect immune cells in tumors by metastasizing to tumors outside the gut, thereby improving the effectiveness of overall immunotherapy. However, it should be noted that there is a clear difference between probiotics and other treatments, such as immunotherapy. Immunotherapy relies on some of the anti-tumor effects of the intestinal flora. Conversely, probiotic treatments involve a direct change to the intestinal microbiome. In healthy people, the use of probiotics mainly plays a role in preventing the occurrence and development of cancer. However, in cancer patients, direct supplementation with probiotics can regulate the intestinal microecology but may have serious adverse effects.

Utilization of precision medicine in different age groups

As the study of gut flora has progressed, researchers have found that there are significant differences in the percentage and abundance of various gut flora in people at different ages. Such a discovery is important for improving the age utilization of precision medicine in gut microbiology. It is specifically categorized into the following areas.

Microbiome studies at different ages

The intestinal flora differs significantly at different ages. Bifidobacteria are the most abundant bacteria in the early stages of life, while *Lactobacillus* and *Escherichia coli* begin to increase with age into adulthood, but bifidobacteria continue to play an important role in healthy populations. In addition, in healthy adults, 80% of the fecal flora were identified as belonging to the following three groups: Bacteroidetes, Bradyrhizobium and Actinobacteria. In the elderly, there was a significant decrease in the number of bifidobacteria, while there was an increase in certain *Clostridium* spp. and Enterobacteriaceae, which have been identified as being detrimental to health (103). Thus microbiome studies can be conducted for different age groups to better understand the relationship between microbes and health and disease. This will help to provide more precise microbiological diagnosis and treatment programs for different age groups.

Development of age-appropriate technologies for the detection of gut microorganisms

There are several emerging technologies that can be used for the detection of gut microorganisms, such as 16S rDNA sequencing (104), Macro-genome sequencing technology (105), Fluorescence quantitative polymerase chain reaction (qPCR) technology (106) and biochip technology (107) among others. Each of these techniques has its own advantages, disadvantages and scope of application, and appropriate methods can be selected for the detection and analysis of intestinal flora according to the specific research objectives and experimental conditions. It is important to point out that for different age groups, such as infants, young children, adolescents, adults, and the elderly, we should actively develop and select the microbiological techniques suitable for their respective characteristics. For example, non-invasive, rapid and accurate testing techniques can be developed for infants and young children to minimize discomfort and risk during the testing process.

Development of personalized gut microbiology diagnostic and treatment protocols

Based on the characteristics of the gut microbiome of people of different ages, combined with information on

the individual's genetic background, lifestyle habits and environmental factors, personalized microbiology diagnostic and treatment protocols will be developed. This will help improve the age utilization of precision medicine in gut microbiology.

Strengthening interdisciplinary cooperation

Encourage researchers in the fields of microbiology, medicine, bioinformatics, etc. to conduct interdisciplinary cooperation to jointly promote the research and application of precision medicine in the field of gut microbiology. By integrating knowledge and technology from different disciplines, a more comprehensive understanding of the relationship between microorganisms and health can be achieved, and more precise diagnosis and treatment services can be provided for different age groups.

In conclusion, to improve the age utilization of precision medicine in microbiology, we need to start from several aspects, including microbiome research for different age groups, development of microbiological testing technologies applicable to different age groups, development of personalized microbiological diagnosis and treatment plans, strengthening of interdisciplinary cooperation, as well as strengthening policy support and regulation. This will help to provide more accurate and personalized microbiology diagnosis and treatment services for different age groups, and improve the age utilization rate of precision medicine in the field of microbiology.

Conclusions

We are encouraged by the emerging findings, but we are also aware of some problems with the current research. First, the process of injecting tumor cells during the establishment of tumor models in mice is equivalent to vaccination, which may cause changes in the immune system. Additionally, factors such as carcinogenic effects and inflammatory stimulation are missing between the artificially created tumor environment and the natural tumor formation process. Second, researchers tend to focus on the effect of gut bacteria on tumors and ignore other biological factors, such as fungi and viruses. Thus any conclusions need further verification to confirm their reliability. Moreover, in practice, it is difficult to extrapolate results from mice to humans. TLRs are indispensable for innate immunity in symbiotic bacterial recognition. However, differences in TLR expression patterns between humans and mice mean that the lymphocytes involved in immunity differ (108). There is still a long way to go before the experimental conclusions drawn from experiments with animals can be applied to humans, and a unified standard for the classification of responders and non-responders in studies is needed in the course of experiments. In addition, researchers should control the average baseline differences measured by influencing factors, such as age, environment, gender, lifestyle, and health status, within a reasonable range to provide criteria for future studies. As an emerging treatment method, ICIs have created more treatment options for cancer patients. However, due to the instability of their structure, ICIs are often affected by multiple factors. Studies on intestinal flora in cancer treatment have shown that clinicians should not only comprehensively evaluate their patients' intestinal microecology in cancer diagnosis and treatment but also carefully and strictly use drugs, such as antibiotics, to avoid destroying the normal balance of the intestinal microenvironment. It is further suggested that clinicians try to use probiotics to maintain and improve the gut microbiota, thereby preventing cancer. Regulating the gut microbiota or adding probiotics may benefit immunotherapy and, eventually, even destroy tumor cells during carcinogenesis.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-316/coif). The authors have no conflicts of interest to declare.

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aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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