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Lower Gastrointestinal Cancers (AB Benson, Section Editor)

Cancer Immunotherapy: Fecal Microbiota Transplantation Brings Light

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Abbreviations *ICIs* Immune checkpoint inhibitors · *PD-1* programmed death receptor 1 · *PD-L1* programmed death-ligand 1 · *CTLA-4* cytotoxic T lymphocyte antigen 4 · *irAEs* immune-related adverse events · *FMT* fecal microbiota transplantation · *PRRs* pattern recognition receptors · *PAMPs* pathogen-associated molecular patterns · *TLRS* toll-like receptors · *NLRs* NOD-like receptors · *APCs* antigen-presenting cells · *IECs* intestinal epithelial cells · *SCFAs* short-chain fatty acids

Opinion statement

Immunotherapy is revolutionizing tumor treatment by activating the immune response to tumors. Among them, immunotherapy represented by immune checkpoint inhibitors is considered to be a milestone in tumor treatment. It has revolutionized the management of advanced malignant tumors by activating T cells, promoting cytotoxic signaling pathways, and killing tumor cells, effectively improving the overall survival of patients. However, resistance to immunotherapy and immune-related adverse events remain challenges for immunotherapy. It has been demonstrated in previous studies that modulating intestinal microbiota can enhance immunotherapy response and reduce complications. Currently, the more mature method for microbiota regulation is fecal microbiota transplantation, which involves transfering a donor's microbiome to the recipient in the form of capsules or fecal microbiota suspension to restore the richness of the recipient's intestinal microbiota. In terms of cancer immunotherapy, fecal microbiota transplantation in patients who fail to

respond to immune checkpoint inhibitors is expected to produce better prognosis for patients.

Introduction

The rise of immunotherapy has successfully transformed the clinical management of advanced malignancies, with immune checkpoint inhibitors (ICIs) in particular playing a key role. Among the most widely used immune checkpoint inhibitors are monoclonal antibodies against programmed death receptor 1(PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T lymphocyte antigen 4 (CTLA-4). At the same time, some new ICIs are also in the early clinical stage, such as lymphocyte activation gene 3 inhibitor, T cell immunoglobulin and mucindomain containing-3 inhibitor and T cell immunoreceptor with Ig and ITIM domains inhibitor [1, 2]. Unfortunately, an increasing number of patients are forced to discontinue ICIs as a result of primary and secondary immune resistance [3]. Due to nonspecific immune stimulation, patients using ICIs are significantly more likely to experience immune-related adverse events (irAEs) [4]. Therefore, immunotherapy resistance and adverse events have also attracted widespread attention [5].

With the new iterations of next-generation sequencing and microbiome technologies, a large number of studies have indicated that the composition of the microbiota has a close association with tumors. It will affect the occurrence, development and response to immunotherapy of tumors, providing new insights for the clinic [6]. The microbiome refers to the genome of the microbial communities (bacteria, fungi and viruses) that inhabit all mucous membranes of the human body [7, 8]. It mainly exists in the intestine and significantly affects the immunity and metabolism of the human body [9]. Recent experiments in animal models by Gopalakrishnan et al. have shown that modulating the intestinal microbiota can increase the sensitivity of solid tumors to ICIs [10]. As of now, fecal microbiota transplantation (FMT) is a promising strategy for reconstructing the new intestinal microbiota by transplanting the functional microbiota in the feces of healthy individuals [11]. However, its impact on immunotherapy is unclear. It is expected that adjusting the intestinal microbiota and optimizing immunotherapy will enhance the quality of life for patients with advanced malignant tumors and prolong their survival.

Immune checkpoint inhibitors usher in a new era in oncology

Compared with traditional tumor treatment methods (such as surgery, radiotherapy and chemotherapy), cancer immunotherapy has significantly improved survival time for patients with metastatic cancer, as well as reducing the rate of recurrence, which has broad prospects. Cancer immunotherapy is an emerging field that aims at restoring the immune system's ability in order to recognize and destroy tumor cells in a natural way. In the past 5 years, immunotherapy has progressed rapidly, especially the application of ICIs like anti-PD-1/PD-L1 and anti-CTLA-4 which is the most important scientific breakthrough to date. The main purpose of ICIs is to overcome the immunosuppressive microenvironment rather than directly enhance immune activity [12].

A type I transmembrane protein known as PD-1 is highly expressed by the T and regulatory T cells as well as by the natural killer cells [13]. PD-1, which is on the surface of T cells, binds to PD-L1 on tumor cells, inhibiting PI3K/AKT and

RAS/MEK/ERK signaling pathways while also suppressing T-cell activity [14, 15]. In addition, the PD-1/PD-L1 pathway also inhibits the proliferation of T lymphocytes and the secretion of cytokines such as IL-2, which will reduce the efficacy of immune cells and promote the escape of the tumor immune system [16]. Similarly, CTLA-4 is also a type I transmembrane glycoprotein that can compete with CD28 for binding to CD80/CD86. But CTLA-4 has a greater affinity for CD80/CD86. Once CTLA-4 binds to CD80/86, it can reduce the costimulatory signal received by T cells and negatively regulate the immune response [13, 17]. As mentioned above, the PD-1/PD-L1 inhibitor is designed to promote immune normalization by blocking the combination of PD-L1 on the surface of tumor cells, along with PD-1 on T cells. It is also beneficial to restore the immune killing function of T cells and kill tumor cells. The CTLA-4 inhibitor activates TCR by blocking the combination of CTLA-4 and CD80/CD86, resulting in T cells proliferating in large numbers and attacking tumor cells [17].

Nowadays, numerous preclinical and clinical studies have shown that targeting CTLA-4 and PD-1/PD-L1 axis can attack tumor cells and enhance antitumor effects [18]. In a preclinical model, after injecting anti-CTLA-4 antibodies into mice with colon cancer, the researchers found that the tumor burden of the mice was significantly reduced, and some mice were even cured, showing a durable survival rate. This study is the first to demonstrate that CTLA-4 inhibitors have significant anti-tumor effects [19]. Additionally nivolumab monotherapy which is an antitumor drug targeting PD-1, improved survival for patients with advanced melanoma compared to dacarbazine according to a phase III clinical trial [20]. In the current state of medicine, nivolumab is approved as a treatment for advanced melanoma. Notably, combination therapy may show higher clinical benefits than anti-PD-1/PD-L1 or anti-CTLA-4 monotherapy. In a retrospective study of advanced melanoma patients treated with nivolumab and ipilimumab, 63% of patients survived three years, which is the longest survival rate so far [21]. Currently, immunotherapy has been approved by the Food and Drug Administration as a treatment for a variety of cancer types, including advanced melanoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma, head and neck squamous cell cancer, refractory classical Hodgkin lymphoma, and urothelial carcinoma [22].

Toxicity of immune checkpoint inhibitors

Unfortunately, with the widespread use of ICIs, there has been a marked increase in the incidence of immune-related adverse events (irAEs) in patients. The common irAEs include rash, colitis, hepatitis, pneumonia, arthritis, and endocrine dysfunction, involving almost all organ systems in the body. It has been reported that more than 80% of patients will experience irAEs [23], more than one third of patients were forced to stop immunotherapy due to severe toxicity, and 1.3% of patients even died [24]. Based on a meta-analysis, it appears that the combined use of nivolumab and ipilimumab may lead to a higher risk of full-grade immune-related endocrine disease than either treatment alone [25]. In the absence of treatment for these adverse reactions, immunotherapy will fail and even put the patient's life at risk. Therefore,

studying the molecular mechanisms leading to irAEs is the focus of our future research.

Notably, only a small proportion of patients respond to ICIs. Resistance to monotherapy has also become an important barrier to treatment, leading patients to be forced to discontinue the drug. A clinical trial in advanced melanoma showed a response rate(RR) of 33.7% of patients receiving pembrolizumab and 11.9% of patients receiving ipilimumab. After 7.9 months of follow-up, 10.6% and 12.1% of responding patients developed successively acquired resistance [26]. Studies have shown that the immunosuppressive microenvironment is one of the main reasons that mediate resistance to immunotherapy [27]. However, due to the complex drug resistance mechanism, there is no standardized clinical treatment plan at present [28].

In light of this, efforts have been made to develop novel immunotherapy combination strategies to overcome potential adverse events and treatment resistance, such as ICIs combined with radiotherapy [29], targeted therapy [30] and fecal microbiota transplantation [31, 32••], etc. There is expected to be an improvement in antitumor efficacy as well as a reduction in toxicity.

Intestinal microbiota and metabolites are associated with immune response

As third-generation sequencing technology has become more widely available, microbiomics has become a hot area of cancer research in recent years [33]. The microbiome refers to the genome of the microbiota distributed on the surface of the human epithelium and mucosa [8]. Studies have shown that the intestinal tract is the main distribution organ of the human microbiota [34]. As a key component of the immune system, the intestinal microbiota interacts with the host cells and is inextricably linked to human health and disease. It is responsible for directing the normal development of the immune system and for innate and adaptive immune functions [35].

As part of innate immunity, pattern recognition receptors (PRRs) are responsible for recognizing pathogen-associated molecular patterns (PAMPs). Microbial PRRs directly stimulate intestinal immunity and increase the expression of toll-like receptors (TLRS), NOD-like receptors (NLRs), and AIM2-like recepotors. As a result, pro-inflammatory factors are released and inflammation is induced by activating the NF-κB signaling pathway [36, 37•] (Fig. 1). Han et al. isolate Lactobacillus rhamnosus HDB1258 from the feces of breastfed infants and examine its effects on innate immune responses. Experiments show that oral administration of HDB1258 in mice can enhance the immune response by activating macrophages and NK cells to initiate innate immunity. Furthermore, it has the ability to promote the expression of anti-inflammatory factors (IL-10), as well as pro-inflammatory factors (TNF-α) to maintain a balance in the immune system [38].

It has also been found that PAMPs from the intestinal tract have the ability to directly interact with immune cells to trigger the activation and maturation of antigen-presenting cells (APCs), which in turn activate B cells and T cells and participate in adaptive immunity [39] (Fig. 1). Specific intestinal microbiota can also influence the development of immune cells. For example, segmented

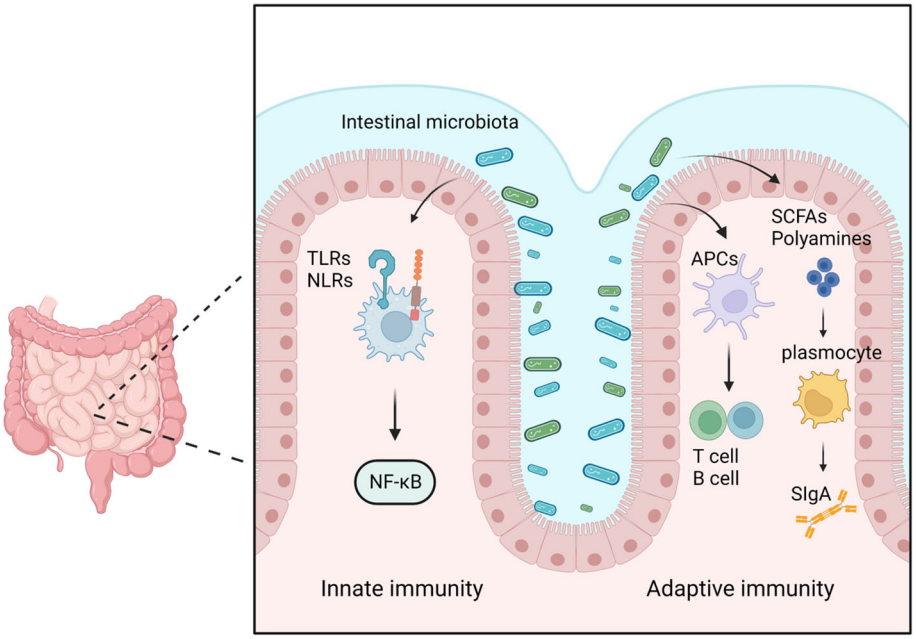

Fig. 1. Intestinal microbiota is associated with immune metabolism. (1) Microbial PRRs directly stimulate intestinal immunity and increase the expression of TLRS and NLRs. The NF-κB signaling pathway then can be activated. (2) In response to PAMPs, APCs are activated and matured, resulting in B lymphocytes and T lymphocytes becoming activated. (3) The intestinal microbiota can also influence the immune system by releasing SCFAs and polyamines. This can lead to the secretion of intestinal IgA as well as the maintenance of the integrity of the intestinal barrier.

filamentous bacteria can adhere to intestinal epithelial cells (IECs) in the ileum and utilize microbial adhesion-triggered endocytosis to transfer T cell antigens to IECs and induce the secretion of Th17 cell [40]. Likewise, Clostridium can promote the differentiation of CD4+ Treg cells [41]. Large numbers of infiltrating Treg cells in the tumor tissue then suppress tumor immunity [42].

The intestinal microbiota can also influence the immune system by releasing metabolites (Fig. 1). For example, among the most abundant microbial metabolites in the intestinal tract are short-chain fatty acids (SCFAs), including acetate, butyrate, and propionate. As inhibitors of histone deacetylases, SCFAs induce a tolerogenic and anti-inflammatory cellular phenotype that is crucial for keeping immune homeostasis [43]. SCFAs have been shown to enhance immunity through the production of IgA by plasma cells, which in turn affects local immunity [44] . Park et al. found that SCFAs are key metabolites that promote the differentiation of naive T cells into Treg cells in the intestinal [45]. Among them, butyrate derived from microorganisms promotes the generation of Treg by inhibiting pro-inflammatory factors from macrophages and inducing the promoter and Foxp3 coding site [46]. Butyrate can also directly enhance the response of CD8+ T cells by modulating the IL-12 signaling pathway [47]. In addition, intestinal microbial metabolites include polyamines, which play a significant role in mucosal immunity by inducing the secretion of intestinal IgA as well as the maintenance of the integrity of the intestinal barrier [39].

All of the above processes suggest that the intestinal microbiota and its metabolites are involved in the immune of the organism, producing a powerful

immune response that is essential for maintaining the immune homeostasis of the organism.

The impact of intestinal microbiota on immunotherapy

It has been found in recent clinical studies that intestinal microbiota can modulate the immune response, which can influence immunotherapy of tumors as a potential therapeutic approach [48]. According to Sivan et al., mice from JAX and TAC laboratories, which have different microbiota in their intestines, developed melanoma at different rates. It was found that the tumor growth rate of TAC mice was the faster between them. The researchers transferred fecal suspensions from JAX mice to TAC mice and found that tumor growth was significantly slowed in TAC mice, suggesting that intestinal microbiota in JAX mice can inhibit tumor growth. The researchers also treated TAC mice with a combination of fecal suspensions from JAX mice and PD-L1 monoclonal antibody. According to the results, this combination significantly improved tumor control and was superior to treatment of ICIs alone [49].

In recent clinical research, the focus has shifted to the specific composition of microbiota that affects immunotherapy in response to the development of new fecal microbial sequence technology. It has been demonstrated that altering the composition of intestinal microbiota may affect immunotherapy toxicity and efficacy. As a result of quantitative metagenomics using shotgun sequencing or 16S rRNA sequencing, the researchers were able to analyze the composition of the intestinal microbiota as well as identify the types of microbiota which are beneficial to immunotherapy based on the clinical benefit of patients [10, 50, 51]. According to Gopalakrishnan's findings, there was a significant difference in the diversity of intestinal microbiota between patients who responded to anti-PD-1 treatment, and those who did not respond. Clostridium, Ruminococcus, and fecal bacteria were found in relative abundance among those who responded, but the diversity was notably reduced among those who did not respond [10]. Matson V et al. found that patients who responded to anti-PD-1 therapy were more abundant in Bifidobacterium longum, Aerobacter, and Enterococcus faecalis. In addition, reconstitution of germ-free mice with fecal material from these patients improved tumor control and enhanced the efficacy of anti-PD-1 therapy [50]. Similarly, Routy et al. transplanted the fecal microbiota of PD-1 responders into germ-free or antibiotic-treated mice, which also improved antitumor effects, whereas the transplantation of non-responders failed to produce results. Meanwhile, metagenomics of fecal samples from patients showed that the clinical response of ICIs was related to the relative abundance of Akkermansia muciniphila [51]. Therefore, a favorable intestinal microbiota composition in the clinic will make ICI therapy more effective.

Aside from improving the efficacy of immunotherapy, the microbiome may also play an important role in reducing immunotherapy-related adverse effects [52]. It has been found that firmicutes was associated with a high incidence of adverse reactions to immunotherapy, while Bacteroidetes was associated with a low incidence of adverse reactions [53]. In a prospective study, Dubin et al. sequenced stool samples from melanoma patients treated with ipilimumab. According to the results, patients with more Bacteroidetes had a lower risk of

colitis [54]. Interestingly, the findings in hepatobiliary tumors contradicted the above. In an analysis of 65 patients with advanced hepatobiliary tumors who received anti-PD-1 therapy, Mao et al. found that 57 of them were suffering from mild colitis, while 8 patients suffered from severe colitis. According to further analysis of patients' intestinal microbiota, patients with severe colitis had higher levels of Bacteroidetes while those with mild colitis had higher levels of firmicutes. It appears that firmicutes play a major role in the prevention of irAEs associated with hepatobiliary tumors based on the results of the study [55].

Based on the above reports, altering the composition of intestinal microbiota can improve the treatment and reduce the toxicity of ICIs, which play a regulatory role in cancer immunotherapy and serve as a potential therapeutic target. As of now, the current research focus needs to be further clarified in order to understand the mechanisms involved in intestinal microbiota and tumor treatment. It is expected that future research results can maximize the effect of immunotherapy.

The application of fecal microbiota transplantation in immunotherapy

It has been demonstrated in previous studies that dietary interventions, probiotics or prebiotics along with other methods have been effective in altering the composition of the intestinal microbiota, with the aim of improving cancer treatment response [56]. However, the most direct approach to microbiota modulation is through FMT. FMT is to isolate fecal microbiota from the feces donated by healthy people, and transplant it into the patient's intestine in the form of bacterial liquid or capsule by perfusion or oral administration to replace the unhealthy intestinal microbiota in the patient [11] (Fig. 2). Currently, a national guideline has recommended FMT for patients with relapsed/refractory Clostridium difficile infection (CDI) [57]. Several studies have confirmed that FMT is also effective in other diseases such as inflammatory bowel disease, irritable bowel syndrome and hepatic encephalopathy [58–60]. Recently, fecal microbiota transplantation has been reported to produce a response in patients who fail to respond to treatment with ICIs, thus making FMT a promising new approach in oncology treatment.

Preclinical mouse models demonstrate that fecal microbiota transplantation can modulate the sensitivity of patients with malignant tumors to ICIs by altering the tumor microenvironment and enhancing tumor immunogenicity. As a result of transplanting fecal microbiota from patients who had responded to anti-PD-1 treatment, mice were more responsive to anti-PD-1 treatment and had a higher density of CD8+ T cells following treatment. Conversely, mice developed resistance to PD-1 after receiving stool from non-responsive patients [10]. At the same time, a clinical study of ICI-related colitis reported that after FMT treatment in patients with colitis, immune cell subsets in the colon were improved and symptoms were relieved [61]. These findings highlight that FMT can improve the response of patients receiving ICI therapy, providing a new therapeutic opportunity for patients with advanced cancer who are refractory to or less effective in immunotherapy, which has promising therapeutic potential.

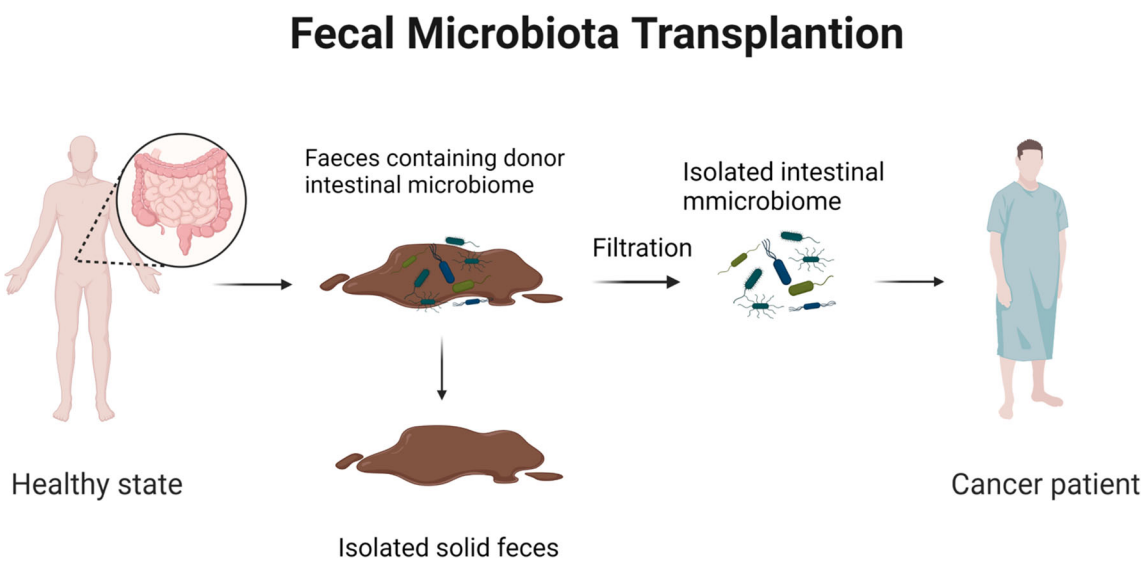

Fig. 2. Fecal Microbiota transplantation FMT is to isolate fecal microbiota from the feces donated by healthy people, and transplant it into the patient's intestine in the form of bacterial liquid or capsule by perfusion or oral administration to replace the unhealthy intestinal microbiota in the patient.

Currently, there are clinical research reports that the combined use of FMT and ICI is not only a more effective treatment, but also has a better safety profile. In the study by Baruch et al., 10 patients with metastatic melanoma that was resistant to anti-PD-1 therapy were treated with combination therapy. Having destroyed their own intestinal microbiota with vancomycin and neomycin, patients receive feces in the form of oral or enteroscopic injection of fecal fluid to re-establish a new intestinal microbiota. At the same time, the patients were treated with anti-PD-1. It was found that two patients had partial remissions, one patient had a complete remission, and the progression-free survival time was more than 6 months for all three patients. Only some patients experienced abdominal distension and other slight discomfort after treatment, with no other adverse effects [62••]. Baruch et al. showed that FMT in combination with ICIs had a profound impact on the treatment of refractory melanoma. After treatment, patients were able to overcome resistance to anti-PD-1 therapy.

Another study, which involved 15 patients with metastatic melanoma resistant to anti-PD-1 therapy, also used a therapy combining FMT and monoclonal antibodies against PD-1 to treat their disease. The results showed a total of 6 patients re-responded to anti-PD-1 and went into remission. Also, 72.9% of treated patients had mild Grade 1 irAEs, and only 3 patients reached Grade 3 irAEs. According to this study, the combination therapy inhibits myeloid-induced immunosuppression, activates CD56+CD8+ T cells in the tumor microenvironment, and reduces IL-8 expression [32••]. Therefore, the rational application of FMT and ICIs in the clinic will produce a better prognosis for cancer patients. Currently, numerous clinical trials are needed to identify specific groups of microbiota that are critical to overcome immunotherapy resistance. Here, we have summarized the clinical trials of FMT combined with ICIs in cancer treatment in recent years (Table 1).

Table 1. Clinical trials of FMT combined with ICIs

NCT number	Title	Status	Cancer types	Interventions	FMT Administration Route
NCT05273255	Fecal microbiota transplantation in patients with malignancies not responding to immune checkpoint inhibitor therapy	Recruiting	Stage IV cancer	Biological: fecal microbiota	
	transplantation (FMT)		Colonoscopy	NCT04988841	Assessing the tolerance and clinical benefit of fecal
	transplantation in patients with melanoma	Recruiting	Melanoma	Drug: MaaT013 Drug: Ipilimumab Drug: Nivolumab Drug: MoviPrep Drug: Normacol Drug: Placebo of Maat013	Capsule
NCT04951583	Fecal microbial transplantation non-small cell lung cancer and melanoma	Recruiting	Non-small-cell lung cancer metastatic advanced melanoma	Combination product: FMT + ICI	Capsule
NCT04924374	Microbiota Transplant in Advanced Lung Cancer Treated With Immunotherapy	Recruiting	Lung cancer	Dietary supplement: microbiota transplant plus anti PD1 therapy Drug: anti PD1 therapy	Capsule
NCT04758507	Fecal microbiota transplantation to improve efficacy of immune checkpoint inhibitors in renal cell carcinoma	Recruiting	Renal Cell Carcinoma	Biological: donor FMT Other: Placebo FMT	Colonoscopy followed by capsules
NCT04729322	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	Recruiting	Metastatic Colorectal		
	Adenocarcinoma		Procedure: Biopsy	Colonoscopy followed by	

Table 1. (Continued)

NCT number	Title	Status	Cancer types	Interventions	FMT Administration Route
	Metastatic Small Intestinal Adenocarcinoma Stage IV Colorectal Cancer AJCC v8 Stage IV Small Intestinal Adenocarcinoma AJCC v8 Stage IVA Colorectal Cancer AJCC v8 Stage IVB Colorectal Cancer AJCC v8 Stage IVC Colorectal Cancer AJCC v8		Procedure: Fecal Microbiota Transplantation Drug: Fecal Microbiota Transplantation Capsule Drug: Metronidazole Drug: Neomycin Biological: Nivolumab Biological: Pembrolizumab Other: Questionnaire Administration Drug: Vancomycin	capsules	
NCT04521075	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 Transplantation by capsules	Recruiting Capsule	Melanoma Stage IV Unresectable Melanoma NSCLC Stage IV	Biological: Fecal Microbial	
NCT04130763	Fecal microbiota transplant (FMT) capsule for improving the efficacy of anti-PD-1	Recruiting	Gastrointestinal System Cancer	Biological: FMT capsule	Capsule

It is undeniable that some cancer patients have individual drug resistance to immunotherapy as well as poorer prognosis due to irAEs. Therefore, alleviation of irAEs is also an option to improve the prognosis of cancer patients with immunotherapy. As mentioned above in the clinical trials, the model of FMT combined with ICIs is still in the initial exploration phase. When it comes to improving irAEs, Wang et al. are pioneers of the therapy, who are the first to report the successful treatment of ICI-associated colitis with FMT [63••]. The result revealed that the patients all had complete clinical relief which returned to normal bowel motility with no further bleeding after FMT. Endoscopic assessment also illustrated significant improvement with reduced inflammation and regression of ulcers. It suggests that FMT has a bright future in the mitigation or treatment of irAEs. We have summarized all the researches published in recent years related to the alleviation of immunotherapy resistance or irAEs with FMT, which provide a certain degree of basis for the application of intestinal microbiota in cancer immunotherapy (Table 2).

Table 2. Clinical applications of FMT combined with ICIs

Cancers	Beneficial Intestinal Microbiota	ICIs	Efects on response/toxicity	Immune regulations	mPFS/PFS	Laboratory
Melanoma	Lachnospiraceae Veillonellaceae Ruminococcaceae	Nivolumab	Diminishing the risk of irAEs (grade 2-4)	Activating Th 1 cells and cytotoxic CD8+ T cells	>6m	Baruch et al. [62••]
Melanoma	Lachnospiraceae Ruminococcaceae Bifidobacteriaceae Coriobacteriaceae	Pembrolizumab	Overcoming resistance to anti PD-1	Increasing activation of CD56+CD8+ T and MAIT cells	3m	Davar et al. [32••]
Urothelial carcinoma	Akkermansia muciniphila	Nivolumab Ipilimumab	Diminishing the risk of ICIs-induced colitis	Inducing the proliferation of CD4+/CD8+ T cells	1m	Wang et al. [63••]
Prostate cancer	Bifidobacterium Blautia	Ipilimumab		Increasing CD4+FoxP3+ cells	>3m	
Gastric cancer	Firmicutes	Pembrolizumab	Diminishing the risk of ICIs-induced colitis	Recovery of bowel movements frequency (case report)	1m	Fasanello et al. [64]
NSCLC	Akkermansia muciniphila	Anti PD-1	Enhancing PD-1 blockade effect	Accumulating CXCR3+ CD4+ T cells	>3m	Routy et al. [51]
Melanoma	Faecalibacterium	Anti PD-1	Up-regulation of PD-L1 in the tumor microenvironment	Increasing CD45+ immune and CD8+ T cells	—	Gopalakrishnan et al. [10]

The limitations of fecal microbiota transplantation in immunotherapy

It is worth noting that the limitations of FMT in cancer immunotherapy should not be overlooked. There is a significant risk of infection when passing donor fecal microorganisms to the recipient. In previous clinical trials, two patients developed broad-spectrum β-lactamase *E. coli* bacteremia after receiving FMT, and one of them died [65]. Currently, the world COVID-19 pandemic also raises questions and concerns about the safety and risks of FMT. Even asymptomatic infected donors can carry the virus in their stool to infect recipients [66]. Therefore, health departments and regulatory agencies should strengthen the safety monitoring and screening of donors.

In addition, another limitation is the use of antibiotics in pretreatment. Prior to combined use of FMT and ICIs treatment, patients first take oral antibiotics to destroy harmful intestinal microbiota in their bodies. It has been reported that vancomycin-neomycin is an effective pretreatment regimen [67]. However, the underlying mechanisms of antibiotic and immunotherapy are not known. Multiple studies have shown that patients who did not receive antibiotics within 1–3 months before immunotherapy and had a rich fecal microbiota tended to respond better to ICIs [68]. Thus, consistent with the results of several clinical trials and conventional treatment, antibiotic exposure may reduce overall survival in patients receiving immunotherapy [69, 70]. For example, in a prospective study, Pinato et al. compared survival in 26 cancer patients who received antibiotics 30 days before ICIs with 151 who did not. The results showed that among patients treated with antibiotics, 81% had disease progression and 8% responded to ICIs. In contrast, 44% of patients who did not receive antibiotics had disease progression and 42% responded to ICIs [71]. Therefore, the use of antibiotics before ICIs treatment often leads to disease progression and patients exhibit poor survival. This important finding suggests that the effect of antibiotic use in the combination of FMT and ICI on clinical response is expected to be further investigated.

The combination therapy of FMT and ICIs has been shown to be feasible. These findings support the idea that resistance to immunotherapy can be overcome through modulating the intestinal microbiota, but the safety deserves some thought. Valuable lessons will be learned from these initial trials and will be used for further optimization in an effort to improve treatment outcomes and guide clinical practice.

Conclusion

Immunotherapy resistance and immune-related adverse events are the biggest obstacles to current cancer immunotherapy. However there has been considerable evidence from preclinical and clinical studies demonstrating the importance of regulating intestinal microbiota in overcoming immunotherapy resistance and reducing immune-related adverse events. FMT is the most direct way

to change the intestinal microbiota and the combined application of FMT and ICIs has also shown good efficacy in clinical trials. However, FMT must follow its ethical principles. The safety of FMT should be the primary consideration in clinical decision-making. Therefore, it is necessary to conduct more clinical studies in order to ensure its safety and efficacy. We look forward to the wide application of FMT in clinical practice in the future to benefit more patients with malignant tumors.

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Author contribution

JZ drafted the manuscript in detail. JZ researched the literatures and have drawn figures. JZ and KW counted and plotted the diagram and table. CS and GL critically revised the article for important intellectual content. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest
The authors declare no competing interests.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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