Gut Microbiota Interventions to Enhance Immune Checkpoint Inhibitor Therapy Clinical Applications Challenges and Future Perspectives

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Abstract. Immune checkpoint inhibitors (ICIs) have emerged as a critical component of cancer immunotherapy, yet the limited response rates and immune-related adverse events (irAEs) continue to restrict their efficacy and safety. The gut microbiota, increasingly recognized as an important regulator of host immunity, has demonstrated its potential in shaping systemic antitumor responses and influencing sensitivity to immune checkpoint blockade. This paper focuses on gut microbiota-targeted interventions as a strategy to enhance ICI efficacy, with particular emphasis on fecal microbiota transplantation (FMT) and probiotics. Recent studies from both experimental models and early clinical trials suggest that such interventions are associated with improved immunotherapy responses and enhanced clinical outcomes in combination with ICIs. Although different in form, FMT and probiotic-based strategies converge on the concept that reshaping the intestinal microbial environment may help overcome suboptimal responses to ICIs. At the same time, the clinical applicability of these approaches is still under active investigation. Overall, this paper summarizes current progress in gut microbiota modulation for cancer immunotherapy, discusses existing limitations, and outlines key challenges for future research. A clearer understanding of how microbiota-targeted interventions interact with ICI therapy is of great importance for their rational integration into clinical practice.

Keywords: Gut microbiota; immune checkpoint inhibitors; fecal microbiota transplantation; probiotic; cancer.

1. Introduction

Cancer has long been a major threat to human health, imposing a severe global disease burden. Projections from the International Agency for Research on Cancer (IARC) indicate that global cancer incidence is expected to reach approximately 35.3 million new cases and 8.5 million deaths by 2050, reflecting a marked rise relative to the 19.3 million cases and 10 million deaths reported in 2020 and underscoring the need for more effective cancer therapies [1]. Presently, commonly applied cancer treatments include surgery, chemotherapy, radiotherapy and immunotherapy. Immunotherapy, particularly immune checkpoint inhibitors (ICIs), was recognized as one of the top ten scientific breakthroughs of 2013 by science. Compared with the tissue damage caused by traditional treatments to normal tissues, ICIs achieve long-term antitumor effects by precisely regulating the immune response, significantly prolonging the survival of patients with advanced tumors. Over the past decade, ICIs have demonstrated effective clinical efficacy by blocking immune checkpoints and reactivating the body's antitumor immune response. As ICIs have become a core treatment for advanced tumors, their efficacy limitations and toxic side effects are even more urgent to address. Among these immune checkpoints, programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1) and cytotoxic T lymphocyte protein 4 (CTLA-4) are the most widely targeted inhibitory surface molecules. Currently, various ICIs have been approved by the Food and Drug Administration (FDA) and have greatly improved outcomes in treating advanced malignancies, such as melanoma and renal cell carcinoma (RCC). However, numerous clinical studies have shown that only 20-40% of patients show clinical responses to ICI therapy, and more than half may develop primary and acquired resistance [2]. Furthermore, about 40% of patients can suffer from immune-related adverse events (irAEs),

which are local or systemic inflammation caused by off-target activation of the immune system [2]. These side effects often interrupt the efficacy of immunotherapy and considerably limit the clinical application and long-term development of ICIs.

To overcome this dilemma, researchers have begun to explore regulatory factors beyond the tumor microenvironment. In 2015, a landmark study by Sivan *et al.* first revealed the key association between the gut microbiota and ICI efficacy, opening a new direction for this field [3]. This study found that the combination of oral administration of *Bifidobacterium* and anti-PD-L1 therapy significantly suppressed tumor growth by boosting dendritic cell (DC) activation and $CD8^+$ T cell infiltration. Another contemporary parallel study also showed that the anti-CTLA-4 therapeutic efficacy is influenced by the microbiota composition [4]. In germ-free or broad-spectrum antibiotic-treated mice, CTLA-4-mediated antitumor responses were substantially attenuated but were re-established through oral administration of *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, or *Burkholderia cepacia*. Currently, multiple mechanisms by which the gut microbiota modulates antitumor immunity have been identified, including activation of pattern recognition receptors (PRRs) to enhance DC function, activation of specific T cells through molecular mimicry, and regulation of the immune microenvironment by microbial metabolites, demonstrating high value in enhancing the efficacy, outcomes, and safety of the ICI treatment. Collectively, these mechanistic insights indicate that deliberate modulation of the gut microbiota represents a viable avenue for enhancing the therapeutic efficacy of ICIs. Among the available strategies, interventions that are clinically implementable have gained particular attention.

Several emerging intervention strategies have been developed to alter gut microbiota, among which FMT and probiotics are the most promising approaches. Various preclinical and clinical trials have demonstrated the significant effect of these translational methods. This paper systematically reviews the application status of gut microbiota-targeted interventions in improving ICI efficacy, deeply analyzes the advantages and limitations of each strategy, and discusses the core challenges of current clinical transformation and future research directions.

2. FMT as a Strategy to Modulate ICI Response

FMT is a direct method to modulate the gut microbiome. Through clinically established oral and endoscopic delivery routes, FMT can transfer feces from the donor to the patient, replacing the unhealthy intestinal microbiota in the recipient. Originally, FMT was developed to treat recurrent Clostridium difficile infections, and two relevant therapeutic products have been approved by the FDA, showing high treatment success rates. Furthermore, several studies have demonstrated the beneficial role of FMT in other diseases, such as metabolic syndrome and multiple sclerosis. FMT has gained attention in cancer immunotherapy as a potential means of augmenting ICI efficacy, given the established role of the gut microbiota in regulating systemic antitumor immune responses and modulating treatment sensitivity. Notably, two core characteristics of FMT-based ICI enhancement strategies have been revealed by preclinical and clinical studies: donor selection and microbiota integrity, which are consistently verified across different cancer types.

2.1. Melanoma

One study compared two genetically similar mice, Jackson Laboratory (JAX) and Taconic Farms (TAC) mice, and found large differences in their gut microbiome and melanoma growth rate [3]. The TAC mice, which exhibited relatively more aggressive tumors, also showed worse efficacy with anti-PD-L1 monoclonal antibody (mAb) treatment alone compared with the JAX mice. Moreover, oral gavage of fecal material from JAX mice into TAC recipients resulted in pronounced tumor growth inhibition, comparable in magnitude to the effects of systemic anti-PD-L1 therapy. As expected, the combination of both JAX fecal administration and ICI therapy in TAC mice displayed the best treatment efficacy. This study further implicated *Bifidobacterium*, particularly *Bifidobacterium bifidum*, as a key contributor to the enhanced antitumor immune responses observed in JAX mice,

with bacterial supplementation resulting in superior tumor control compared with unsupplemented controls. This study provided the first experimental evidence linking gut microbiota and immunotherapy together and pointing out the potentially critical role of *Bifidobacterium* in enhancing the efficacy of anti-PD-L1 treatment in melanoma. This relationship was further substantiated by findings indicating that CTLA-4-mediated antitumor responses were shaped by specific members of the *Bacteroides* genus [4]. In a cohort of 25 patients with metastatic melanoma (MM) receiving ipilimumab, comparative assessment of pre- and post-treatment gut microbiome composition identified three clusters (A, B, and C). An increase in patients with cluster C was observed after ipilimumab treatment and the fecal transplantation of cluster C into germ-free mice markedly improved the response to CTLA-4 blockade and effectively suppressed the tumor growth. Notably, cluster C-recipient mice exhibited outgrowth of *Bacteroides fragilis*, which was not observed in cluster A- or cluster B-recipient mice.

Later, 2 clinical trials on MM were the landmark studies analyzing how FMT treatment can affect anti-PD-1 immunotherapy, testing its safety and feasibility. In a phase 1 clinical trial (NCT03353402), 10 recipients with MM refractory to anti-PD-1 therapy were enrolled and 2 MM patients who had achieved durable complete responses (CR) to PD-1 blockade treatment for at least 1 year were chosen to be donors [5]. After antibiotic pretreatment, recipients underwent FMT by colonoscopy and oral fecal capsules, together with the reinduction of nivolumab. 3 patients achieved objective responses (ORs), including 1 CR and 2 partial responses (PRs). All responders experienced progression-free survival (PFS) beyond 6 months, as assessed by iRECIST criteria. Although the remaining patients did not meet criteria for OR, immunologic changes were noted in some cases. Overall, the observed response rate in this heavily pretreated, immunotherapy-refractory population exceeded expectations from PD-1 re-challenge alone, supporting a potential clinical benefit of FMT in enhancing immunotherapy efficacy. Another phase 2 clinical trial (NCT03341143) evaluated the effect of FMT in overcoming resistance to PD-L1 blockade treatment [6]. In this study, patients with MM who exhibited primary resistance to anti-PD-1 treatment were administered fecal microbiota obtained from donors with durable OR to PD-1 blockade. The results demonstrated that 3 out of 15 patients achieved ORs, including 1 CR and 2 PRs, and 3 additional patients experienced durable stable disease lasting more than 12 months. All responders showed improved PFS and overall survival (OS) compared with nonresponders. Together, these 2 trials in patients with anti-PD-1-refractory MM established proof-of-concept support for FMT as a strategy to reverse resistance to immunotherapy.

Further studies on the treatment efficacy of FMT from healthy individuals combined with ICI therapy were performed. In a cohort of 20 previously untreated MM patients, Routy *et al.* investigated the use of FMT from healthy donors alongside PD-1 blockade with nivolumab or pembrolizumab [7]. This phase 1 trial (NCT03772899) reported that the OR rate was up to 65% (13 of 20), including 20% CRs, and additional patients achieved disease stabilization lasting over 6 months, resulting in a 75% clinical benefit rate. Among responding patients, FMT was associated with increased representation of immunogenic taxa, including *Ruminococcus, Faecalibacterium*, and *Eubacterium*, accompanied by a reduction in potentially deleterious bacteria such as *Enterocloster*. Moreover, the Avatar mouse models in this study further confirmed the effective role of stools from both healthy donors and responders in increasing anti-PD-1 efficacy. To summarize, melanoma studies confirm that FMT enhances ICI efficacy through enriching immunogenic bacteria, and donor selection and microbiota remodeling are key factors. The evidence chain has expanded from refractory patients to treatment-naive populations, supporting its broad clinical potential.

2.2. RCC

Several preclinical and clinical studies in RCC have been conducted, shedding light on the important role of FMT. Researchers reported that the use of broad-spectrum antibiotics (ampicillin + colistin + streptomycin) can markedly shorten the PFS and OS of RCC patients who received ICI therapy [8]. In addition, *Akkermansia muciniphila* was found to be the most significant taxon differing between responders and nonresponders to PD-1 mAb therapy. In this study, stool samples from 7

RCC patients, including both responders and nonresponders, were introduced into antibiotic-preconditioned BALB/c mice, which were then orthotopically engrafted with PD-1-refractory renal tumor cells and administered combined CTLA-4 and PD-1 blockade. The results revealed that FMT from clinical responders restored the antitumor efficacy of dual checkpoint blockade, whereas FMT from nonresponders failed to confer such a benefit. In a phase 2 clinical trial (NCT03013335), 69 RCC patients who received nivolumab therapy were prospectively involved, and the results demonstrated that the recent antibiotic use substantially reduced the OR rate, PFS, and OS, suggesting that antibiotics compromised the efficacy of ICIs [9]. Moreover, FMT from 5 responders and 10 nonresponders was performed in the RCC-bearing mice, and the finding was consistent with the previous study, revealing that fecal microbiota from responding RCC patients can improve ICI efficacy while microbiota from nonresponders would even attenuate its antitumor effect. Importantly, this study found that administration of FMT from responders or responder-enriched immunostimulatory commensals, such as *Akkermansia muciniphila* or *Bacteroides salyersiae*, successfully overcame resistance induced by FMT from nonresponders and restored ICIs sensitivity.

Fernandes *et al.* conducted a phase 1 clinical study (NCT04163289) to evaluate the safety and feasibility of administering FMT prior to first-line ICI-based therapy among RCC patients, as well as its ability to mitigate irAEs. Data from the enrolled 10 patients showed that 93.3% of planned FMT procedures were delivered, and the OR rate achieved 44% (4 of 9), including 3 CRs. Although irAEs occurred in 80% of patients, including grade 3/4 events in 60%, no FMT-related dose-limiting toxicities were observed. Another phase 2 clinical trial (NCT04758507) was the first randomized study assessing the ability of FMT to potentiate first-line VEGFR-TKI plus ICI therapy in RCC [10]. The results demonstrated that transferring stool from a patient with a complete and durable ICI response significantly enhanced treatment outcomes. Compared with the placebo group, FMT markedly improved the 1-year PFS rate (66.7% vs. 35.0%), extended median PFS (14.2 vs. 9.2 months), and increased the OR rate (54% vs. 28%), with an acceptable safety profile. Across both clinical trials, administering responder- or healthy individual-derived FMT prior to immunotherapy was safe and demonstrated the potential to enhance the antitumor efficacy of ICIs in RCC, highlighting FMT as a promising microbiome-based therapeutic strategy.

3. Probiotic-Based Approaches to Potentiate ICI Therapy

Probiotics represent a targeted microbiota modulation strategy that complements FMT. Unlike FMT, which replaces the entire gut microbiota, probiotics achieve ICI enhancement by delivering specific live beneficial bacteria, with clearer active components and lower safety concerns. Probiotics have been increasingly recognized for their health-promoting effects, and in the context of cancer immunotherapy, strains of *Bifidobacterium* and *Lactobacillus* have been shown to reshape the gut microbiota and potentiate responses to ICIs.

3.1. *Bifidobacterium* spp.

The genus of *Bifidobacterium* contains various probiotics, widely applied in intestinal health products. After analyzing the stool samples from 96 NSCLC patients, including 54 responders and 42 nonresponders, researchers found that responders were characterized by higher levels of *Bifidobacterium bifidum*, whereas nonresponders showed relative enrichment of *Akkermansia muciniphila* and *Blautia obeum* [11]. The mouse tumor models further revealed that only specific strains of *B. bifidum* (*B. bif*_K57, K18, and M31) enhanced the tumor-reducing activity of anti-PD-1 compared with anti-PD-1 alone. Notably, the synergistic effect extended to a syngeneic 4T1 breast cancer model, which is typically resistant to PD-1 blockade, and although neither B. *bif*_K57 nor anti-PD-1 was effective individually, their combination significantly suppressed tumor growth. In a randomized phase 1 clinical trial (NCT03829111), Dizman *et al.* evaluated whether the bifidogenic live bacterial product CBM588 could augment the efficacy of nivolumab plus ipilimumab in treatment-naive metastatic RCC [12]. The results reported that the addition of CBM588 resulted in

markedly prolonged PFS (12.7 vs. 2.5 months) and a higher OR rate (58% vs. 20%). Moreover, *Bifidobacterium* was found to be enriched in responders due to CBM588, indicating the critical role of bifidogenic shifts in shaping a microbiome configuration that supports more effective immunotherapy.

3.2. *Lactobacillus* spp.

Lactobacillus is another common type of probiotic. In a murine model with established tumors, oral delivery of *Lactobacillus reuteri* significantly limited tumor progression and prolonged survival [13]. It further demonstrated that *L. reuteri* could translocate to, and colonize in melanoma, secreting indole-3-aldehyde (I3A), which is a kind of dietary tryptophan catabolite. The released I3A strongly augmented the efficacy of ICIs by promoting interferon-γ-producing $CD8^+$ T cells. The viability of *L. reuteri* was confirmed to be both necessary and sufficient to improve the antitumor effect of ICIs in melanoma. Another study established a mechanistic basis for utilizing live *Lactobacillus rhamnosus* GG (LGG) as an oral adjunct to enhance ICI efficacy. In murine models of CRC and melanoma, the combination of live LGG and PD-1 mAb synergistically inhibited the tumor growth through reshaping the gut microbiota toward immunostimulatory species and promoting tumor infiltration of DCs and $CD8^+$ T cells. All these findings highlighted probiotics as promising microbiota-based interventions capable of potentiating ICI efficacy.

4. Cross-Species Common Mechanisms and Future Perspectives

Integrating the above evidence from FMT and probiotic studies, the core mechanism of microbiota modulation enhancing ICI efficacy can be summarized as microbiota-immune axis regulation: immunogenic bacteria or their metabolites promote the activation of $CD8^+$ T cells and DCs, as well as the tumor infiltration of $CD8^+$ T cells, while suppressing deleterious bacteria reduces immune suppression in the tumor microenvironment. These mechanisms appear to be broadly shared across melanoma and RCC, although the dominant bacterial contributors vary by cancer type. These findings collectively highlight both FMT and probiotics as promising microbiota-based interventions capable of potentiating ICI efficacy, and future research should focus on three directions to advance their clinical application: establishing personalized microbiota modulation strategies based on cancer type and individual patient microbiota profiles, exploring the combination of FMT and probiotics to achieve overall microbiota remodeling and precise beneficial strain supplementation, and clarifying the long-term safety and optimal administration timing of such microbiota-targeted interventions.

5. Conclusion

Mounting evidence highlights the intimate link between gut microbiota and host immunity, underscoring the substantial potential of microbiota interventions in addressing the limited response rate, drug resistance and toxicity of current immunotherapies. Preclinical and early clinical studies in MM, RCC and other cancers have reported that microbiota modulation reshapes systemic antitumor immunity and enhances sensitivity to PD-1, PD-L1 and CTLA-4 blockade. The two major intervention strategies, FMT and probiotics, promote the enrichment of immunogenic bacteria and restore antitumor immune responses, accompanied by enhanced DC activation, elevated type I interferons and improved infiltration and effector function of $CD8^+$ T cells. Clinical evidence further confirms that microbiota modulation improves OR rates and prolongs PFS in selected settings, including patients with primary or acquired resistance to ICIs, with acceptable safety profiles. Despite these encouraging outcomes, microbiota-based strategies are still in the early stage of clinical translation, hampered by individual heterogeneity in baseline microbiota, variable microbial engraftment and durability, safety and regulatory concerns, and the lack of effective biomarkers for patient stratification. Future research directions should prioritize the development of personalized strategies for microbiota modulation, integrating multi-omics analyses to identify functional

microbial signatures predictive of ICI responses, and conducting randomized clinical trials to determine optimal intervention timing and long-term safety. These advances will define whether gut microbiota-targeted interventions can serve as a reliable and effective adjunct to cancer immunotherapies.

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