Research progress on remodeling the gut microbiota and the immune microenvironment for the treatment of hepatocellular carcinoma: a post-print

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Date: 2026-02-10T16:48:26+00:00

Abstract

Hepatocellular carcinoma (HCC), as the most common form of primary liver cancer and a leading cause of cancer-related mortality, remains therapeutically challenging due to drug resistance and the low response rate to immune checkpoint inhibitors (ICIs). The gut microbiota regulates the hepatic immune microenvironment via the gut–liver axis and plays a pivotal role in the occurrence, progression, and immunotherapy of HCC. This article systematically and comprehensively explores how the gut microbiota, through its metabolic products and immune signals, affects macrophage polarization, dendritic cell antigen presentation, natural killer (NK) cell cytotoxicity, and T cell function, thereby reshaping the immune microenvironment of HCC. The composition of the HCC immune microenvironment is closely associated with the efficacy of programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) inhibitors, and characteristic microbial taxa may serve as predictive biomarkers of treatment response. In addition, microbiota-targeted interventions such as probiotics, fecal microbiota transplantation, and prebiotics can enhance antitumor immune responses by modulating microbial composition, and have shown the potential to improve the efficacy of ICIs in animal models and preliminary clinical studies. Therefore, the gut microbiota may provide novel targets and strategies for HCC immunotherapy, and future clinical studies are needed to validate its clinical applicability and to optimize patient prognosis. This review offers a reference for the development and application of gut microbiota–related targets and strategies in HCC immunotherapy, and provides insights for designing clinical studies that combine HCC immunotherapy with microbiota interventions to improve patient outcomes.

Full Text

Research Progress on Gut Microbiota Remodeling the Immune Microenvironment in Hepatocellular Carcinoma Treatment

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Abstract

Hepatocellular carcinoma (HCC), a common form of primary liver cancer and leading cause of cancer-related mortality, remains challenging to treat due to therapeutic resistance and low response rates to immune checkpoint inhibitors (ICIs). The gut microbiota modulates the hepatic immune microenvironment through the gut-liver axis and plays a pivotal role in HCC initiation, progression, and immunotherapy. This review systematically examines how gut microbiota reshapes the HCC immune microenvironment by influencing macrophage polarization, dendritic cell antigen presentation, natural killer (NK) cell cytotoxicity, and T-cell function through microbial metabolites and immune signaling pathways. The composition of the HCC immune microenvironment is closely associated with therapeutic efficacy of programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors, and specific microbial taxa may serve as predictive biomarkers of treatment response. Additionally, microbiota-targeted interventions such as probiotics, fecal microbiota transplantation (FMT), and prebiotics have shown potential to enhance ICI efficacy in animal models and preliminary clinical studies by modulating microbial community structure and improving antitumor immune responses. Therefore, gut microbiota may offer novel therapeutic targets and strategies for HCC immunotherapy, though further clinical studies are required to validate their clinical applicability and optimize patient outcomes. This review provides a reference for developing and applying gut microbiota-related therapeutic targets and strategies in HCC immunotherapy, and may inform future clinical studies integrating microbiota interventions to improve treatment outcomes in HCC patients.

Keywords: Hepatocellular carcinoma; Gut microbiota; Immune microenvironment; Immune checkpoint inhibitors; Microbiota intervention strategies

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the third leading cause of cancer-related death worldwide [1]. While viral hepatitis-related HCC has decreased in recent years, cases associated with metabolic dysfunction-associated fatty liver disease (MAFLD) and alcohol-related liver disease continue to rise [2]. Despite significant advances in surgical resection, liver transplantation, targeted therapy, and the application of immune checkpoint inhibitors (ICIs), current treatments remain inadequate for meeting the needs of HCC patients. Therefore, novel therapeutic targets and strategies to enhance ICI efficacy are urgently needed.

The gut microbiota comprises a complex and diverse microbial community residing in the intestinal mucosa that is essential for maintaining immune homeostasis and metabolic health [3]. Through the gut-liver axis, gut microbiota regulates hepatic function and immune responses, making it a key participant in HCC development [4]. Studies have reported that gut dysbiosis can rapidly reshape the inflammatory microenvironment, impair antitumor immune surveillance, and promote HCC progression [5]. Furthermore, gut microbiota composition is a critical factor influencing ICI efficacy, as it correlates not only with HCC patient response to immunotherapy but also with the development of drug resistance through modulation of immune cell activity [6]. Given these roles, research has shown that probiotics, fecal microbiota transplantation (FMT), and prebiotics can improve immunotherapy outcomes in HCC by modulating gut microbiota [7-8].

This review examines the effects of gut microbiota on key immune cells in HCC (macrophages, dendritic cells, NK cells, CD4+ T cells, and CD8+ T cells), analyzes the association between gut microbiota and ICI therapeutic efficacy, and explores the potential of microbiota-targeted interventions to improve ICI treatment outcomes. This work provides new insights and strategies for immunotherapy in HCC patients.

Literature Search Strategy

We conducted computerized searches of PubMed, Web of Science, and other databases from inception to July 2025 using English search terms including "Hepatocellular Carcinoma," "Gut Microbiome," "Tumor Microenvironment," "Immune Checkpoint Inhibitors," "Probiotics," "Fecal Microbiota Transplantation," and "Prebiotics." Inclusion criteria comprised basic experimental studies, clinical research, and reviews related to gut microbiota regulation of the immune microenvironment in HCC, ICI efficacy, and microbiota intervention strategies. Exclusion criteria included duplicate publications, irrelevant topics, and inaccessible full texts. A total of 63 articles were ultimately included.

Figure 1

Figure 1: Figure 1

Effects of Gut Microbiota on HCC Immune Cells

Gut microbiota reshapes the tumor immune microenvironment and influences HCC development by regulating the function and quantity of various immune cells, including macrophages, dendritic cells, natural killer (NK) cells, CD8+ T cells, and CD4+ T cells

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Macrophages

The liver is rich in innate immune cells, particularly macrophages, which play a key role in mediating phagocytosis and exerting cytotoxic effects to inhibit intratumoral spread [9]. In MAFLD-related HCC mouse models, *Akkermansia muciniphila* abundance decreases approximately 40-fold during disease progression. Treatment with *A. muciniphila* reduced M2 macrophage numbers while increasing effector memory CD4+/CD8+ T cells in tumors, thereby inhibiting tumor growth [10]. Additionally, *A. muciniphila* ameliorated hepatic steatosis by suppressing cholesterol biosynthesis and bile acid metabolism. Yao et al. [11] found that dextran-carbinoxolone (DEX-CBX) conjugates reduced relative abundance of lipopolysaccharide (LPS)-associated microbiota by 95-45% in an orthotopic HCC mouse model, while increasing *A. muciniphila* abundance approximately 37-fold. DEX-CBX intervention decreased M2 macrophages and significantly increased NKT and CD8+ T cells, suppressing tumor growth. These findings suggest DEX-CBX may exert anti-HCC effects by modulating gut microbiota and altering the immune microenvironment, while alleviating LPS-induced HCC immunosuppression.

Gut dysbiosis can increase M2 macrophages and promote HCC development. Studies have reported significantly elevated interleukin-25 (IL-25) levels in serum and tissues of HCC patients, with high IL-25 expression in HCC tissues correlating negatively with survival after curative hepatectomy [12]. Gut dysbiosis can cause colonic tuft cell hyperplasia, increasing IL-25 secretion. In an orthotopic liver tumor mouse model, IL-25 induced macrophage polarization toward the M2 phenotype, promoting HCC cell migration, invasion, and tumorigenesis. Xia et al. [13] found that gut dysbiosis caused abnormal bile acid (BA) metabolism, which subsequently modulated the tumor immune microenvironment by preventing NKT cell recruitment and increasing M2-like tumor-associated macrophages, thereby promoting tumor immune escape and HCC progression.

In summary, alterations in gut microbiota influence HCC development by affecting the role of M2 macrophages and other immune cells within the immune microenvironment.

Dendritic Cells (DCs)

DCs are among the most potent antigen-presenting cells in the immune system, capable of initiating adaptive immune responses and enhancing innate immunity. Studies show that *Escherichia coli* strain Nissle 1917, a widely used probiotic, stimulates DC activation in HCC mouse models, leading to robust antitumor immune responses [14]. Furthermore, combination therapy with *E. coli* Nissle 1917 and the transforming growth factor-β (TGF-β) blocker Galunisertib triggered tumor-specific effector T cell responses, increased intratumoral interferon-γ (IFN-γ) and CD8+ T cell accumulation, shifted the tumor environment from immunosuppressive to immunostimulatory, and restored TGF-β-blocked cytotoxicity and heat shock response capacity. This changed the tumor microenvironment and enhanced NK cell antitumor responses [15]. These findings indicate that *E. coli* Nissle 1917 can enhance antitumor immunity by activating DCs, suggesting its combination with immunomodulatory drugs may represent a novel strategy for HCC immunotherapy.

NK Cells

NK cells possess antitumor, antiviral, and immunomodulatory functions. Studies report that *Brevibacillus parabrevis* enhances anti-HCC effects by promoting lipid breakdown into acetyl-CoA, which inhibits NK cell ferroptosis. Additionally, this bacterium catalyzes acetylation of RAR-related orphan receptor C (RORC), enhancing its binding to the NEDD4-like E3 ubiquitin protein ligase (NEDD4L) promoter, leading to ferroptosis transporter ubiquitination and degradation, and inducing NK cell differentiation into an adaptive phenotype with enhanced cytotoxicity and heat shock response capacity, thereby altering the tumor microenvironment and enhancing NK cell antitumor responses [15]. Other studies show that *Bacteroides acidifaciens*, *Odoribacter laneus*, and *Odoribacter planchnicus* (collectively termed BOO) enhance NK cell antitumor function in HCC mouse models [16]. BOO also restores NK cell dysfunction caused by gastrointestinal microbiota disruption, further strengthening anti-HCC effects.

Gut microbial imbalance can exacerbate chronic inflammation, increase tumor susceptibility, and impair CD8+ T cell activity. Patients with nonalcoholic fatty liver disease (NAFLD)-related HCC exhibit significant gut microbiota dysbiosis, with composition and function changing during HCC progression. Nonalcoholic steatohepatitis (NASH) patients have an HCC prevalence as high as 38%, significantly higher than other non-cirrhosis etiologies [17]. Li et al. [18] found that *A. muciniphila* abundance was significantly reduced in both NASH-related HCC patients and mouse models. Further research revealed that *A. muciniphila* strain AM06, isolated from breast milk, inhibited NASH-to-HCC progression in mice by increasing CXC motif chemokine receptor 6 (CXCR6)+ NKT cells. In vitro experiments demonstrated that AM06 promoted NKT cell cytotoxicity against liver cancer cells. Studies show that *Bacteroides uniformis* and *Bifidobacterium bifidum* are significantly reduced in NASH patients. Supplementation with these

bacteria not only increased hepatic NK cell numbers and restored their function in NASH mice but also improved liver pathology and metabolic status, thereby delaying NASH progression [19].

Recent studies have found that aldo-keto reductase 1D1 (AKR1D1) deficiency increases *Bacteroides ovatus* proportion, which deoxycholic acid into isolithocholic acid, promoting isolithocholic acid accumulation that impairs NK cell cytotoxic function and accelerates HCC progression. However, spironolactone, a potassium-sparing diuretic, effectively enhances NK cell antitumor immunity and inhibits HCC growth when combined with ICIs by targeting isolithocholic acid (animal study) [20]. This suggests that targeting isolithocholic acid produced by *B. ovatus* may represent a novel approach to activate NK cell cytotoxicity for HCC treatment.

These studies demonstrate that gut microbiota enhances anti-HCC effects and inhibits NASH-to-HCC progression by remodeling NK cell function and increasing their numbers in the immune microenvironment. Conversely, gut microbiota imbalance and related metabolites can cause NK cell dysfunction and accelerate HCC progression.

CD8+ T Cells

CD8+ T cells are key effector cells in antitumor immunity, closely associated with HCC development, progression, and treatment response. Stigmasterol is a compound with antitumor properties that induces tumor cell apoptosis. Huo et al. [21] found that in an HCC mouse model, stigmasterol altered gut microbiota α- and β-diversity and significantly increased abundance of *Lactobacillus johnsonii*, *Lactobacillus murinus*, and *Lactobacillus reuteri*. These microbial increases led to significantly higher numbers of CD8+ T cells in tumors, enhanced immune responses within the host tumor microenvironment, markedly upregulated apoptosis protein expression, and ultimately caused tumor cell death. Other studies show that *Bacteroides thetaiotaomicron*-derived acetic acid can modulate proinflammatory macrophage polarization and promote cytotoxic CD8+ T cell function, thereby enhancing antitumor immunity and inhibiting HCC growth (animal study) [22].

However, gut microbiota-derived metabolites can trigger T cell immunosuppression, manifested by reduced CD8+ T cell expansion (prospective study) [23]. In advanced HCC, sorafenib is an effective first-line therapy, but resistance is becoming increasingly common and is closely related to the tumor microenvironment [24]. A prospective study of advanced HCC patients found that increased IFN-γ+CD8+ T cells correlated with improved survival after sorafenib treatment [25]. Yu et al. [26] showed that four bacterial genera—*Lachnoclostridium*, *Lachnospira*, *Enterobacter*, and *Enterococcus*—were associated with sorafenib efficacy in advanced HCC. *Enterococcus faecium* played an important role in modulating sorafenib activity and was significantly enriched in the gut of advanced HCC patients who responded well to sorafenib. Further studies in subcutaneous

HCC xenograft mouse models demonstrated that *E. faecium* combined with sorafenib enhanced antitumor activity by increasing IFN-γ and CD8+ T cell proportions in the tumor microenvironment. Additionally, *E. faecium*-secreted exopolysaccharides stimulated intratumoral IFN-γ production by CD8+ T cells, which then synergized with sorafenib to induce ferroptosis in HCC cells.

In summary, gut microbiota enhances tumor immunity against HCC by increasing CD8+ T cell quantity and function, as well as enhancing sorafenib efficacy.

CD4+ T Cells

CD4+ T cells coordinate humoral and cellular immunity by secreting cytokines that promote immune cell activation [27]. T helper 17 (Th17) cells are an important CD4+ effector T cell subset with proinflammatory properties that maintain chronic inflammatory states and create a pro-tumorigenic microenvironment [28]. Th17 cell percentages are significantly elevated in peripheral blood of HCC patients, with high Th17 levels predicting poor prognosis [29].

Li et al. [30] found that the probiotic mixture Prohep suppressed tumor growth in an HCC mouse model by altering gut microbiota, promoting anti-inflammatory bacterial proliferation, and reducing Th17 cell levels. *A. muciniphila* and *Bifidobacterium bifidum* prevented NAFLD progression in mice by activating hepatic farnesoid X receptor (FXR), suppressing intestinal FXR expression, modulating gut microbiota, and improving intestinal mucosal permeability, thereby reducing Th17 cells and proinflammatory cytokine levels [31]. These findings suggest gut microbiota may influence NAFLD-to-HCC progression by modulating Th17 cell levels. Therefore, reducing Th17 cell levels within CD4+ T cell subsets through gut microbiota modulation may represent a novel strategy for HCC intervention.

Gut Microbiota and Efficacy of Immune Checkpoint Inhibitors

PD-1 and PD-L1 Inhibitors

PD-1/PD-L1 are important immune checkpoint molecules. PD-1/PD-L1 inhibitors restore antitumor immune responses by releasing tumor cell-mediated T cell suppression and have become crucial for advanced HCC treatment. Zheng et al. [32] analyzed dynamic changes in gut microbiota composition and characteristics in sorafenib-refractory HCC patients receiving anti-PD-1 immunotherapy. Results showed non-responders had increased Proteobacteria beginning at week 3, which became dominant by week 12, while responders had greater gut microbial diversity with enrichment of *A. muciniphila* and Ruminococcaceae (prospective study). Another study of 65 advanced hepatobiliary cancer patients receiving anti-PD-1 therapy found that clinical benefit group had higher abundance of *Alistipes sp. Marseille* P5997 and *Lachnospiraceae bacterium* GAM79 compared to the no clinical benefit group, with longer overall survival (OS) and

progression-free survival (PFS). Conversely, patients with high Veillonellaceae abundance showed poorer PFS and OS (prospective study) [33]. A study analyzing gut microbiomes of 45 unresectable HCC patients receiving anti-PD-1 combination therapy found significant β-diversity differences between responders and non-responders. Patients with high abundance of *Collinsella*, *Ruminococcus*_AM4211, and *Ruminococcus*_AF25_{28}AC had longer median PFS and OS, while those with high *Bacteroides*_AF20_{13}LB and *Veillonella atypica* had significantly shorter median PFS and OS (prospective study) [34]. Other clinical studies have confirmed differences in gut microbiomes between responders and non-responders in HCC patients receiving PD-1/PD-L1 inhibitors (prospective studies) [35-36].

Additionally, a study of eight advanced HCC patients receiving nivolumab found that responders had higher Shannon diversity indices with distinct microbial community structures. Non-responders showed marked dysbiosis, while patients with higher Prevotella/Bacteroides ratios responded better to nivolumab, highlighting the potential of gut microbiome as a prognostic marker for nivolumab response (prospective study) [37]. However, this study was limited by small cohort size and insufficient statistical power. These preliminary data should be interpreted cautiously, with future larger studies likely revealing additional microbial biomarkers.

In summary, gut microbiome may influence the efficacy of PD-1/PD-L1 inhibitor immunotherapy in HCC patients. Characteristic gut microbiome compositions can serve as effective biomarkers for predicting immunotherapy response and survival benefit, providing new therapeutic targets for HCC patient response to immunotherapy.

CTLA-4 Inhibitors

Ponziani et al. [38] studied 11 HCC patients receiving the CTLA-4 inhibitor tremelimumab and/or durvalumab, finding that responders had higher relative abundance of *Akkermansia* and lower Enterobacteriaceae compared to non-responders (prospective study). Another study showed that depletion of beneficial gut microbiota Firmicutes and Bacteroidetes in HBV-HCC patients was associated with enhanced CTLA-4-mediated immunosuppression (cross-sectional observational study) [39].

These findings suggest that specific gut microbiota signatures (e.g., high *Akkermansia* abundance, low Enterobacteriaceae, and high Firmicutes/Bacteroidetes abundance) may alleviate CTLA-4-mediated immunosuppression and enhance CTLA-4 inhibitor efficacy. However, whether gut microbiota changes directly affect CTLA-4 inhibitor efficacy requires further investigation.

Microbiota Intervention Strategies

Probiotics

Probiotics are live microbial supplements that confer health benefits to the host by improving gut microbial balance. In mouse models, oral *Bifidobacterium* alone controlled tumor growth comparably to PD-L1-specific antibody therapy, while combination treatment nearly eliminated tumor growth through enhanced DC function and CD8+ T cell accumulation in the tumor microenvironment [40]. Gao et al. [41] found that *Lactobacillus rhamnosus* Probio-M9 improved PD-1 inhibitor antitumor effects in mice by increasing beneficial bacteria abundance and reshaping functional gut metagenomes. Notably, Probio-M9 supplementation significantly increased abundance of *Parabacteroides distasonis* and *Bifidobacterium pseudolongum*. *P. distasonis* abundance correlates with antitumor immunotherapy effectiveness and may predict response to PD-1 and CTLA-4 therapy [42], while *B. pseudolongum* can enhance immunotherapy response through its metabolites [43]. These findings demonstrate that probiotic administration can improve efficacy and response to anti-PD-1/PD-L1 immunotherapy. Although these studies were not conducted in HCC models, they demonstrate the potential of probiotics to enhance PD-1/PD-L1 inhibitor antitumor effects through gut microbiota modulation, supporting the feasibility of exploring probiotic formulations combined with PD-1/PD-L1 inhibitors for HCC treatment.

Recent literature reports that tumor-suppressing multi-enterobacteria (TSME), a mixture of nine probiotic strains, improved outcomes in anti-PD-L1 (atezolizumab)-resistant advanced HCC patients when used in combination, achieving approximately 7 months PFS with long-term survival [44]. In HCC tumor-bearing mice, TSME combined with anti-PD-1 therapy enhanced therapeutic efficacy by increasing CD8+ and CD4+ T cell proportions while reducing regulatory T cell (Treg) proportions in the tumor microenvironment. The relative tumor inhibition rate reached 58.78% ± 7.55% in the TSME plus anti-PD-1 combination group. Additional clinical trial data show that probiotic supplementation combined with PD-1 inhibitors and antiangiogenic agents yields longer PFS in advanced HCC patients [45]. However, clinical effects of probiotic preparations in antitumor immunotherapy vary, necessitating consideration of probiotic strain types, specifications, and mechanisms of action to achieve reliable outcomes.

These findings provide theoretical support for clinical application of probiotics combined with HCC immunotherapy. Several clinical trials are currently investigating this approach: NCT05620004 is exploring *Bifidobacterium* as a tumor immunomodulator in HCC patients [46]; NCT05032014 is recruiting 46 HCC patients to evaluate the impact of *L. rhamnosus* Probio-M9 on PD-1 inhibitor treatment response [47]; and NCT06551272 is assessing the probiotic EXL01 containing an unmodified *Faecalibacterium prausnitzii* strain in HCC patients receiving PD-L1 immunotherapy [48] (Table 1).

Fecal Microbiota Transplantation (FMT)

FMT involves transferring fecal microbiota from healthy donors to recipients to restore gut microbial balance and treat intestinal and extraintestinal diseases. A clinical trial of patients receiving ICIs combined with FMT showed that ICI responders exhibited increased abundance of microbiota associated with anti-PD-1 response, enhanced CD8+ T cell activation, and reduced myeloid cells expressing proinflammatory interleukin-8 (IL-8) [49]. This demonstrates that FMT combined with anti-PD-1 can alter gut microbiome and reprogram the tumor microenvironment. Gut dysbiosis interferes with antitumor treatment efficacy. Li et al. [50] performed FMT using fecal samples from HCC patients and healthy individuals in an HCC mouse model, finding that dysbiotic microbiota from HCC patients impaired antitumor immune responses by suppressing antigen presentation and effector T cell function through the cGAS-STING-IFN-I pathway.

Although FMT combined with ICIs has not been fully validated in HCC clinical studies, significant differences in gut microbiota composition between ICI responders and non-responders in HCC patients suggest that FMT could enhance immunotherapy efficacy by altering gut microbial composition. This indicates FMT combined with ICIs may be a promising HCC treatment strategy, offering new directions for improving immunotherapy outcomes through gut microbiota modulation. Clinical trials of FMT in HCC treatment remain limited. Trial NCT05690048 is evaluating whether FMT can overcome resistance to atezolizumab/bevacizumab in HCC [51]. Similarly, a phase II trial (NCT05750030, completed recruitment) is assessing FMT safety in HCC patients who responded to atezolizumab/bevacizumab and those who did not respond to prior immunotherapy [52] (Table 1).

Prebiotics

Prebiotics are macromolecules that selectively enhance the metabolism and proliferation of beneficial gut probiotics, thereby improving host health. Liu et al. [53] found that bilberry anthocyanins increased gut microbiome species diversity, elevated fecal butyrate concentration and proportion, and enhanced intratumoral CD8+ T cell infiltration, thereby improving anti-PD-L1 efficacy in a colon cancer mouse model. Recent research suggests that the prebiotic lactitol may promote proliferation of *Megasphaera* and *Bifidobacterium*, leading to longer OS in HCC patients (retrospective study) [54]. Although insufficient evidence currently confirms that prebiotics can improve HCC immunotherapy outcomes through gut microbiome modulation, their potential warrants attention. Future clinical trials assessing specific prebiotic interventions combined with ICIs could provide evidence for clinical application.

Potential Risks and Challenges

Probiotic intervention may pose safety concerns in immunocompromised patients who are more susceptible to infection, requiring stringent safety screening and monitoring of probiotic strains [55]. Probiotics may also trigger hyperactive immune responses, increasing autoimmune disease risk [56]. One study showed that *Bifidobacterium longum* or *Lactobacillus rhamnosus* GG intervention reduced PD-1 inhibitor therapeutic response in melanoma mouse models, with lower IFN-γ and CD8+ T cell numbers in the tumor microenvironment [57]. Thus, probiotic intervention may carry risks of promoting tumor progression.

A multicenter phase I trial found that incorporating FMT into anti-PD-1 therapy did not increase immune-related adverse event (irAE) incidence [58]. However, despite its potential, FMT carries significant risks. Complete transfer of donor microbiota may disrupt the recipient' s existing microbial balance. In two independent clinical trials, two patients developed drug-resistant *E. coli* bacteremia after FMT, with genomic sequencing confirming both cases were linked to the same fecal donor, resulting in one fatality [59]. These cases underscore the need for enhanced donor screening to limit potential infectious events and maintain vigilance regarding FMT benefits and risks across different patient populations. Additionally, standardized and broadly applicable FMT protocols with appropriate donors are needed to minimize FMT-associated risks [60]. Recent literature reports that regional microbial implantation mismatches after FMT may negatively impact host metabolism and immunity, altering fundamental properties of regional gut ecosystems [61]. This emphasizes the importance of precise matching between specific regional gut microbiota and their corresponding microenvironments.

The complexity and individual specificity of gut microbiota present challenges for microbiota intervention strategies. Each individual' s gut microbial community is unique, shaped by genetic background, diet, living environment, and lifestyle habits, which complicates development of universal microbiota modulation therapies. Moreover, gut microbiota is highly dynamic, with continuous structural evolution over time and in response to external stimuli (e.g., medications, dietary changes). HCC patients exhibit particularly complex and variable gut microbiota compositions [62]. Variations in fecal collection, storage, and processing protocols during clinical microbiota studies may introduce bias and reduce reproducibility [63], further complicating standardization of probiotic, FMT, and prebiotic interventions and posing challenges for achieving stable, reproducible therapeutic efficacy.

Summary and Outlook

In summary, gut microbiota plays a crucial role in regulating the HCC immune microenvironment through the gut-liver axis. Through metabolites, immune molecules, and signaling pathways, gut microbiota influences key immune cells including macrophages, DCs, NK cells, and T cells, reshaping the immune mi-

croenvironment and participating in HCC development. Gut microbiota composition is closely correlated with ICI (PD-1/PD-L1, CTLA-4 inhibitors) efficacy, and characteristic gut microbiota signatures can serve as biomarkers for ICI therapy response. Additionally, microbiota-targeted strategies including probiotics, FMT, and prebiotics have demonstrated potential to enhance immunotherapy efficacy in animal models and preliminary clinical studies, providing feasible approaches to improve patient survival and quality of life. However, several challenges remain: probiotics carry risks of infection and hyperimmune responses; FMT faces donor safety and regional microbiota implantation mismatch issues; and the individual specificity, dynamic nature, and standardization difficulties of gut microbiota interventions pose challenges for clinical application in HCC immunotherapy.

Although the role of gut microbiota in HCC immunity and immunotherapy remains incompletely understood, with current research focusing primarily on correlations, these findings present significant opportunities for HCC diagnosis, immunotherapy, and prevention based on gut microbiome. Future research should validate the clinical applicability of gut microbiota modulation in HCC through large-scale, multicenter studies. Identifying effective microbial biomarkers, optimizing patient stratification, and assessing treatment responses are essential for advancing microbiota-based interventions. Additionally, improving targeted microbiota modulation strategies will enhance specificity and controllability. Furthermore, in-depth investigation of microbiota characteristics and treatment response heterogeneity across different HCC etiologies (viral hepatitis, NASH, MAFLD) is needed. Ultimately, the goal is to improve HCC immunotherapy outcomes and optimize patient prognosis through precise gut microbiota modulation.

Author Contributions: Li Xiangxiang and Liu Zhongyu contributed to conception and design, literature collection and organization, and manuscript writing. Zhang Zhaojie, Zhai Yujie, and Li Honglin contributed to manuscript revision, quality control, and review. Yu Xiaohui contributed to final version revision and overall responsibility for the article.

Conflicts of Interest: None declared.

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TABLE:1 Clinical Trials on Microbiota Intervention Strategies Combined with HCC Immunotherapy