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REVIEW

How the gut microbiome affects the immunotherapy response in hepatocellular carcinoma

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ABSTRACT Hepatocellular carcinoma (HCC) remains a major global health challenge with limited long-term survival despite advances in surgical, locoregional, and systemic treatments. Although immune checkpoint blockade (ICB) has reshaped HCC therapy, only a subset of patients achieves durable responses, reflecting substantial heterogeneity in tumor biology and immune microenvironments. Dysbiosis, involving the loss of beneficial bacteria, like *Lactobacillus reuteri* and *Akkermansia muciniphila*, and the expansion of pathogens, such as *Klebsiella pneumoniae* and *Catenibacterium mitsuokai*, drives HCC by promoting microbial translocation and chronic inflammation. This process is mediated by microbiota-derived metabolites. Pro-carcinogenic agents, like deoxycholic acid (DCA) and quinolinic acid, induce inflammation and activate oncogenic pathways, while protective short-chain fatty acids (SCFAs), like acetate and butyrate, modulate T-cell and ILC3 responses to influence antitumor immunity. Tryptophan catabolites, acting *via* the aryl hydrocarbon receptor (AhR), further fine tune immune and barrier functions. In addition, emerging data implicate intratumoral microbiota as active modulators of immune suppression and metastatic behavior. These mechanistic insights have accelerated the development of microbiome-targeted interventions, such as probiotics, prebiotics, engineered bacterial strains, and fecal microbiota transplantation, to enhance ICB responsiveness. This review synthesizes current advances linking the gut microbiome to HCC immunobiology and highlights emerging therapeutic strategies aimed at optimizing immunotherapy through precise microbial modulation.

KEYWORDS Hepatocellular carcinoma; gut microbiome; immunotherapy; gut–liver axis; microbial metabolites; dysbiosis; intratumoral microbiota; probiotics; fecal microbiota transplantation

Introduction

Hepatocellular carcinoma (HCC) constitutes the principal form of primary liver cancer and represents a significant global public health challenge. According to cancer statistics in 2022 from the World Health Organization (WHO), liver cancer ranks as the sixth most common malignancy in incidence and the third most common cause of cancer-related deaths globally[1]. Major risk-factors for HCC include chronic infection with hepatitis B or C virus, alcoholic liver disease, and metabolic dysfunction-associated steatotic liver disease (MASLD)[2]. The primary therapeutic strategies for HCC in appropriate patients include surgical resection, thermal ablation [such as radio-frequency ablation (RFA)], transarterial chemoembolization (TACE), and transplantation. Small-molecule targeted therapies, such as tyrosine-kinase inhibitors, were approved by the Food and Drug Administration (FDA) as first-line systemic options in advanced disease in 2008[3]. However, high rates of post-treatment recurrence and acquired resistance to chemotherapy and small-molecule targeted agents continues to be a major clinical obstacle. The 5-year survival rates of patients with advanced or unresectable HCC are often ≤10%–20%[4,5]. Therefore, immune-based therapy has emerged as a highly promising new therapeutic paradigm in HCC over the past decade[6-9].

Immune checkpoint blockade (ICB) therapy targets inhibitory receptors, such as programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which tumors exploit to evade immune surveillance. PD-1 signaling suppresses effector T-cell activity within the tumor microenvironment (TME), while CTLA-4 dampens T-cell priming in lymphoid tissues. Antibody-mediated blockade of these checkpoints restores cytotoxic T-cell function and enhances

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anti-tumor immunity. ICB has reshaped systemic therapy among HCC therapeutic strategies (**Figure 1**). Early monotherapies with nivolumab or pembrolizumab achieved objective response rates (ORRs) of 15%–20% but survival benefits were limited[10-12]. The combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) in the phase III IMbrave150 trial established a new first-line standard with a median progression-free survival (PFS) of 6.8 months[7]. Similarly, the STRIDE regimen [single-dose tremelimumab (anti-CTLA-4) plus durvalumab (anti-PD-L1) maintenance] from the HIMALAYA trial improved median overall survival to 16.43 months, marking dual checkpoint blockade as another approved frontline option[8]. More recently, findings from the CheckMate 9DW trial further substantiated the survival advantage conferred by dual ICB, demonstrating that the combination of nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) significantly improves overall survival (OS) in patients with advanced HCC[9].

Despite notable advances in ICB therapy, a minority of HCC patients achieve durable responses, reflecting multiple mechanistic and biomarker-related challenges. Heterogeneous expression of immune checkpoints, particularly PD-L1, limits predictive accuracy[13]. The tumor mutational burden (TMB), while a predictive biomarker in several solid tumors, is generally low in HCC[14,15] and substantial heterogeneity exists across etiologic subtypes, including virus-associated, MASLD-related, and WNT/β-catenin-driven tumors, all of which affect neoantigen load and immune visibility[16]. The tumor immune microenvironment further constrains ICB efficacy because many HCCs are "immune-cold" and characterized by sparse tumor-infiltrating lymphocytes (TILs) and enrichment of immunosuppressive populations, such as regulatory T (Treg) cells, myeloid-derived suppressor cells (MDSCs), and M2-like macrophages[17]. Intrahepatic and inter-subtype heterogeneity further complicates the immune landscape because individual tumor nodules or molecular subclasses exhibit divergent

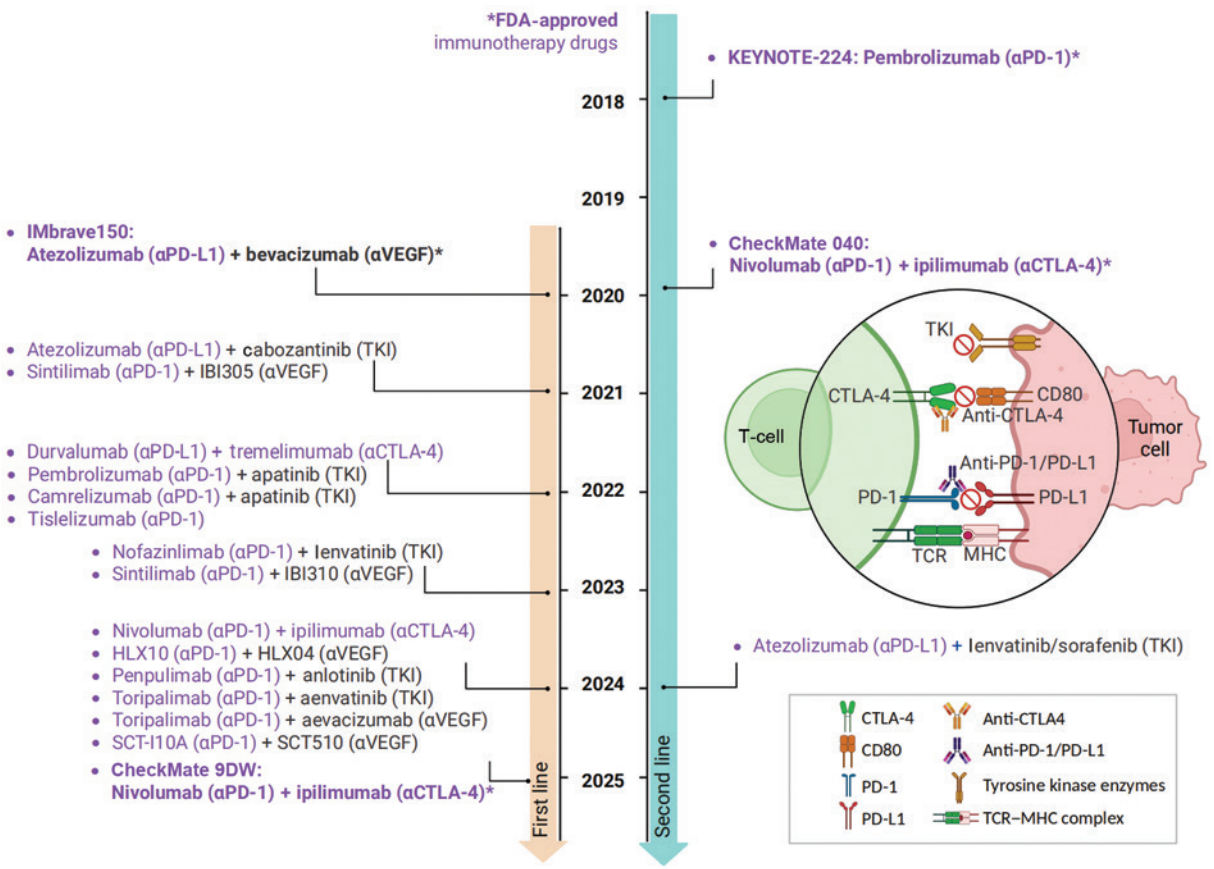


Figure 1 Landscape of immune checkpoint-based systemic therapies for HCC. Timeline summarizing major first- and second-line immune checkpoint-based therapeutic regimens for advanced HCC from 2018 to 2025, including PD-1/PD-L1 inhibitors, CTLA-4 inhibitors, VEGF-targeting antibodies, and other tyrosine kinase inhibitors. FDA-approved immunotherapy combinations are indicated. CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CD80, cluster of differentiation 80 (also known as B7-1); PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TCR, T-cell receptor; MHC, major histocompatibility complex; αVEGF, anti-vascular endothelial growth factor; TKI, tyrosine kinase inhibitor. Figure created using BioRender (www.biorender.com).

immune microenvironments, checkpoint expression profiles, and responses to immunotherapy[18,19]. Thus, integrative strategies that combine multi-dimensional, biomarker-guided approaches are essential to improve response rates and durability in HCC.

The gut microbiome not only regulates local intestinal immunity but also exerts a critical influence on systemic immune regulation in HCC *via* the gut–liver axis, thereby shaping the hepatic immune microenvironment and responses to immunotherapy. Under physiologic conditions, a balanced microbial community maintains intestinal barrier integrity, modulates bile acid and short-chain fatty acid (SCFA) metabolism, and preserves hepatic immune homeostasis[20]. In contrast, dysbiosis, which is characterized by the loss of beneficial taxa, increased gut permeability, and altered metabolite composition, facilitates the translocation of microbial-associated molecular patterns (MAMPs), and secondary bile acids[21,22]. This process drives chronic hepatic inflammation, activates innate immune signaling pathways, and promotes the expansion of immunosuppressive myeloid populations, including MDSCs and M2-polarized macrophages[23]. Notably, specific microbial metabolites, such as deoxycholic acid, have been shown to impair hepatic natural killer T cell recruitment and attenuate antigen presentation, thereby establishing an immunologically "cold" TME that is less responsive to ICB[24,25]. These findings highlight the gut microbiome as a central regulator of hepatic immunity and underscore the potential of the gut microbiome as a biomarker and therapeutic target to enhance immunotherapy efficacy in HCC.

The role of the gut microbiome in HCC development *via* the gut–liver axis

Physiologic connection between the gut and liver

The liver and intestine are intricately linked *via* the gut–liver axis, encompassing the portal vein, bile ducts, and lymphatic vessels. This connection is essential for maintaining metabolic and immune homeostasis. The intestinal epithelium functions as a selective barrier in healthy individuals, permitting nutrient and metabolite absorption, while restricting microbial translocation[26]. This barrier consists of a monolayer of epithelial cells reinforced by tight junctions, adherens junctions, and a mucous layer enriched with antimicrobial peptides and secretory IgA. Commensal microbiota contribute to barrier maintenance by producing SCFAs, particularly butyrate, which enhances epithelial integrity, supports colonocyte energy metabolism, and modulates local immune activity[27]. The gut-associated lymphoid tissue (GALT) provides immunologic surveillance and maintains tolerance to dietary and microbial antigens, while facilitating rapid defense against pathogens[28]. Metabolites and microbial products absorbed in the intestine reach the liver through the portal circulation, where hepatocytes, Kupffer cells, and sinusoidal endothelial cells integrate these signals to coordinate metabolism and immune responses[29-32]. Bile acids secreted by the liver into the intestine reciprocally shape the microbial composition, establishing a dynamic feedback loop that maintains immune equilibrium[33]. This tightly regulated system ensures low-level exposure to microbial components, preventing unwarranted hepatic inflammation and sustaining immune homeostasis.

Microbial dysbiosis and disruption of the gut barrier during HCC development

The gut–liver axis represents a dynamic immunometabolic network and disturbances within this system play a pivotal role in liver disease. The intestinal epithelium and its commensal microbiota maintain mucosal integrity and regulate hepatic immune tolerance through the gut–liver axis under physiologic conditions[34] (**Figure 2**).

Chronic liver disease: gut–liver axis disruption alters the disease trajectory

Progressive disruption of gut microbial homeostasis and intestinal barrier integrity represents a fundamental pathogenic mechanism underlying chronic liver disease. Among hepatic disorders, alcohol-associated liver disease (ALD) was one of the earliest identified conditions in which gut-derived endotoxins and pathogen-associated molecular patterns (PAMPs) were shown to have a causal role[35]. Mechanistic studies demonstrated that ethanol-induced dysbiosis and intestinal barrier damage promote bacterial translocation, endotoxemia, and inflammatory hepatic injury, establishing ALD as a prototypical inflammation-driven disease of the gut–liver axis[36,37]. Excessive alcohol consumption profoundly alters gut microbial composition[38]. Recent studies have further linked depletion of beneficial taxa, such as *Akkermansia muciniphila*, to impaired mucosal integrity and aggravated hepatic injury, while supplementation with this bacterium ameliorates ethanol-induced barrier dysfunction and liver damage[39]. *Enterococcus faecalis* has emerged as another key pathogenic driver. Targeted bacteriophage therapy effectively eliminates these strains in humanized mice and significantly reduces hepatic inflammation and necrosis[40]. Moreover, ethanol exposure suppresses the hepatic complement receptor, compromising bacterial clearance and exacerbating microbial translocation[41]. Alcohol-induced gut dysbiosis and barrier breakdown cooperate to activate hepatic immune signaling, sustain inflammation, and drive progressive liver injury through the gut–liver axis.

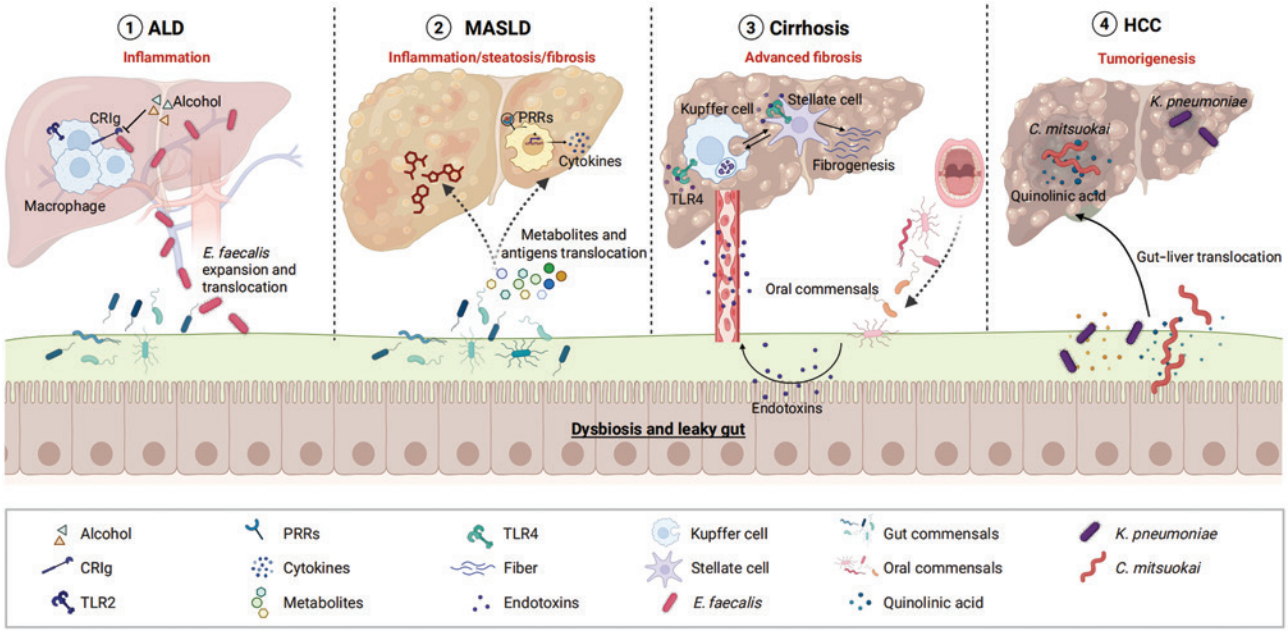


Figure 2 Gut–liver axis dysregulation and microbiome-driven mechanisms across the spectrum of hepatocarcinogenesis. Progressive liver disease, from ALD-to-MASLD-to-cirrhosis, is characterized by dysbiosis, impaired gut barrier integrity, microbial translocation, and chronic inflammation. (1) **ALD**: chronic alcohol use causes an intestinal bloom of the pathobiont, *E. faecalis*, allowing *E. faecalis* to translocate to the liver. Alcohol exposure remodels hepatic macrophages, downregulating CRIg on Kupffer cells and impairing bacterial clearance. The accumulated *E. faecalis* in the liver and blood is sensed by TLR2, which activates inflammatory pathways that drive disease pathology. (2) **MASLD**: The condition is characterized by an altered gut microbiome and dysregulated bile acid metabolism. This dysbiosis, combined with increased intestinal permeability (a "leaky gut"), allows bacterial metabolites and antigens to translocate from the gut into the portal circulation. Once in the liver, these microbial products are detected by PRRs, which initiate inflammatory and stress-response pathways. This cascade of events drives the key pathogenic features of MASLD: hepatic steatosis; inflammation; and fibrogenesis. (3) **Cirrhosis**: Severe dysbiosis and endotoxemia result from loss of barrier integrity and overgrowth of oral commensals, fueling Kupffer-cell activation and stellate-cell-mediated fibrosis through TLR4 activation. (4) **HCC**: Microbial translocation from gut-to-liver introduces tumor-promoting bacteria (e.g., *C. mitsuokai* and *K. pneumoniae*) and metabolites (e.g., quinolinic acid), which foster hepatocarcinogenesis. Together, these processes illustrate how gut microbial alterations and leaky gut drive inflammation, immune dysfunction, and liver cancer progression. ALD, alcohol-associated liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; CRIg, complement receptor of the immunoglobulin family; PRRs, pattern recognition receptors; TLR2, Toll-like receptor 2; TLR4, Toll-like receptor 4. Figure created using BioRender (www.biorender.com).

MASLD is a complex metabolic disorder affecting up to 30% of the global population. Increasing evidence underscores the central role of the gut–liver axis in the pathogenesis of MASLD. Impaired intestinal barrier function and endotoxemia, which were initially observed in type 2 diabetes, are prevalent in MASLD and facilitate microbial translocation and hepatic inflammation[42]. Interestingly, it has been shown that the microbial pattern in MALSD patients is likely alcohol-producing[43]. Dysregulation of lipid metabolism, particularly ceramide synthesis, drives hepatic steatosis and insulin resistance, while commensal bacteria, including *Bacteroidetes* spp., modulate ceramide production, linking the microbiota directly to metabolic homeostasis[44-46]. Gut microbial profiling reveals stage-specific dysbiosis. Advanced fibrosis and cirrhosis are characterized by increased *Proteobacteria* and *Escherichia coli* abundance and depletion of *Firmicutes*, whereas steatosis is associated with lower microbial diversity and enrichment of *Coprococcus* and *Ruminococcus gnavus*[47,48]. Notably, long-term gut microbiome instability correlates with MASLD development and metabolic co-morbidities, suggesting a causal role rather than a mere consequence of diet or metabolic disease[49].

Cirrhosis represents the terminal stage of chronic liver disease and exemplifies the cumulative impact of gut–liver axis disruption. Patients with decompensated cirrhosis exhibit marked intestinal inflammation, epithelial barrier loss, and elevated fecal cytokine concentrations, which are biomarkers that correlate more strongly with disease severity than circulating inflammatory mediators[50]. In patients with liver cirrhosis, gut microbial gene richness is markedly reduced compared to healthy individuals and accompanied by a depletion of beneficial, butyrate-producing taxa, such as

Faecalibacterium prausnitzii, *Coprococcus comes*, and members of *Lachnospiraceae* and *Ruminococcaceae*, indicating loss of a health-associated microbiome. Conversely, cirrhotic patients exhibit enrichment of oral-origin bacteria, including *Campylobacter*, and *Haemophilus parainfluenzae*, suggesting translocation of oral commensals into the gut, likely facilitated by altered bile composition and intestinal permeability[51]. Mechanistically, endotoxin-mediated activation of the TLR4 signaling pathway in hepatic macrophages and stellate cells has a pivotal role in driving hepatic inflammation and fibrogenesis[52]. Collectively, these findings illustrate a mechanistic continuum in which gut microbial dysbiosis and barrier disruption foster endotoxin-mediated hepatic inflammation and fibrosis, ultimately facilitating the transition from cirrhosis to hepatocarcinogenesis.

HCC: gut-liver translocation as a pivotal driver

Emerging evidence indicates that gut microbiota actively shapes the TME in HCC. Tumor-associated microbiota are enriched in *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, with taxa, such as *Ruminococcus gnavus* and *Stenotrophomonas maltophilia*, linked to hepatic stellate cell activation and senescence-associated secretory phenotypes (SASPs)[53,54]. Dysbiosis is characterized by increased *Bacteroides* and *Ruminococcaceae*, reduced *Bifidobacterium*, and altered lipopolysaccharide (LPS)- and butyrate-producing genera in MASLD- or cirrhosis-associated HCC, which correlates with systemic inflammation and hepatocarcinogenesis[55]. Hepatitis B virus (HBV)-related HCC displays enrichment of anti-inflammatory taxa, such as *Prevotella*, *Lactobacillus*, and *Faecalibacterium*, indicating virus-specific modulation of gut-immune interactions[56]. Mechanistic studies have moved beyond correlation to establish causation, demonstrating that gut-derived bacteria can translocate to the liver and directly drive oncogenesis. For example, fecal microbiota transplantation from HCC patients to mice can induce liver disease, a process driven by the pathogen, *Klebsiella pneumoniae*. This bacterium translocates and promotes hepatocarcinogenesis through the surface protein (PBP1B), which activates the TLR4 signaling pathway[57]. A recent study revealed a similar mechanism for *Catenibacterium mitsuokai*, which was shown to be enriched in HCC tumors. This bacterium translocates by binding to γ-catenin on liver cells *via* the Gtr1/RagA protein and secretes quinolinic acid, which activates the TIE2 receptor and downstream PI3K/AKT pathway to drive tumor growth[58]. Both studies provided mechanistic evidence that targeting these specific bacteria or the signaling pathways suppress HCC progression, establishing a direct causal role for the gut microbiota in hepatocarcinogenesis. Collectively, these findings underscored the progressive influence of gut microbiota on liver disease, linking early metabolic dysregulation in MASLD to cirrhosis-associated inflammation and ultimately HCC, and highlighting the gut–liver axis as a potential target for biomarker development and therapeutic intervention.

Microbiome–immune interactions relevant to HCC microenvironment

Gut microbiota-derived metabolites shaping the immune response

Gut microbiota-derived metabolites, particularly bile acid derivatives and SCFAs, constitute critical mediators of host–microbe communication that orchestrate hepatic immune homeostasis and influence the immunopathogenesis of HCC. Among these metabolites, bile acid metabolites have emerged as potent immunoregulatory molecules that bridge metabolic and inflammatory signaling (**Figure 3**).

Bile acids

Primary bile acids synthesized by hepatocytes are transformed by commensal bacteria into secondary bile acids, which can exert profound effects on innate and adaptive immunity[59]. Specific bile acid species modulate the balance between proinflammatory Th17 and anti-inflammatory Treg cells through direct engagement of nuclear receptors, such as RORγt and farnesoid X receptor (FXR)[60,61]. For example, 3-oxo-lithocholic acid (3-oxoLCA) binds RORγt to inhibit Th17 differentiation, while isoallo-lithocholic acid (isoalloLCA) enhances Treg cell generation, collectively contributing to immune tolerance and gut–liver immune equilibrium[60]. Conversely, bile acids accumulation, particularly deoxycholic acid (DCA), promotes chronic hepatic inflammation under dysbiotic or high-fat dietary conditions. DCA activates hepatic stellate cells (HSCs), induces a SASP, and stimulates the production of prostaglandin E2 (PGE2), which suppresses antitumor immune responses and facilitates hepatocarcinogenesis[62]. Some secondary bile acids can further impair enterohepatic circulation and hepatic FXR activation by promoting $CD8^+$ T cell-mediated ileitis through TGR5/mTOR-dependent metabolic reprogramming[63].

Bile acid signaling also modulates innate immune surveillance through receptor-mediated pathways. Activation of FXR and G protein-coupled receptor TGR5 suppresses NF-κB and NLRP3 inflammasome activation, thereby restraining excessive cytokine release and preventing chronic inflammation[64,65]. Inhibition or dysregulation of FXR function disrupts immune homeostasis, enhances intestinal permeability, and favors the proliferation of tumor-promoting microbial species. Moreover, bile acid-dependent chemokine signaling in liver sinusoidal endothelial cells regulates CXCL16-mediated recruitment of natural killer T (NKT) cells, linking microbial metabolism to hepatic immune surveillance and tumor control[24].

SCFAs

Similarly, SCFAs, which are derived from bacterial fermentation of dietary fibers, are major immunomodulatory metabolites influencing gut and hepatic immune tone[66]. SCFAs

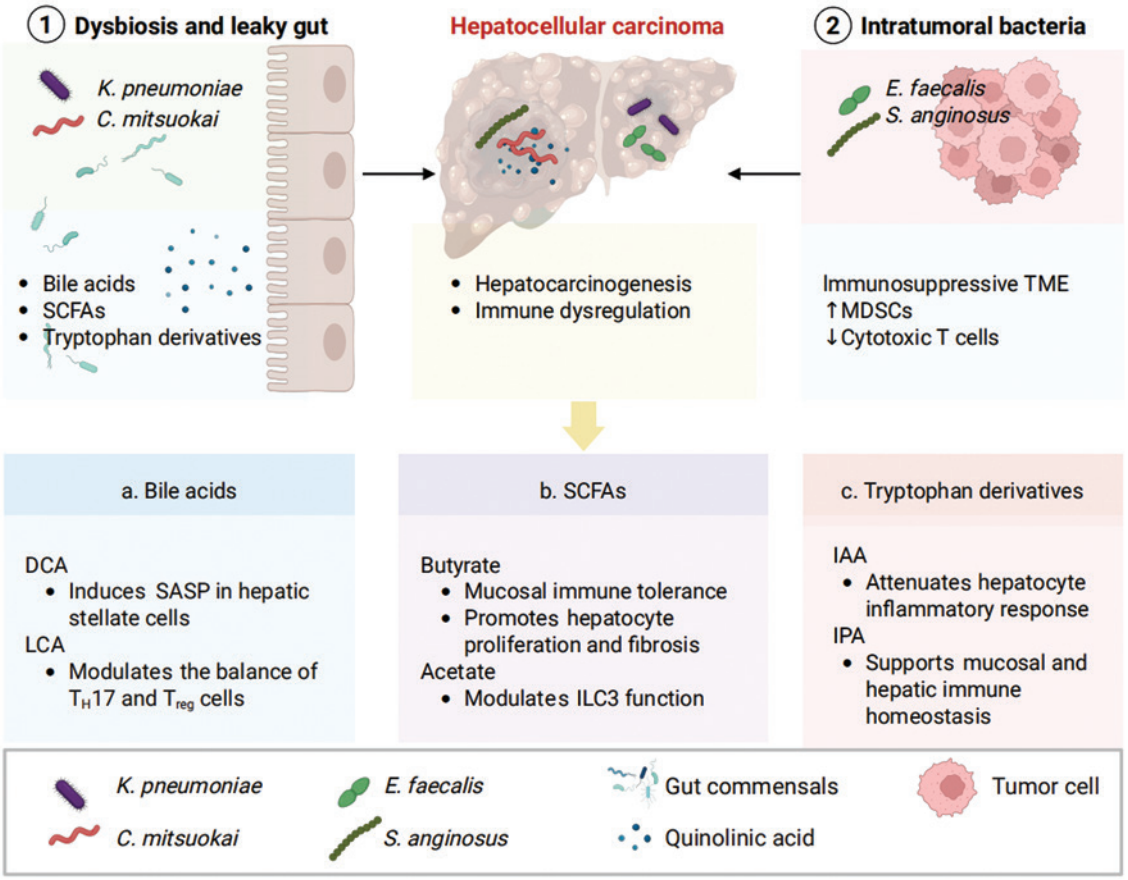


Figure 3 Gut–liver axis and host immune microenvironment in hepatocarcinogenesis. **(1) Dysbiosis and leaky gut:** Dysbiotic gut communities enriched in pathogenic species (e.g., *C. mitsuokai* and *K. pneumoniae*) enable bacterial dissemination and metabolite flux across the gut–liver axis, contributing to hepatocarcinogenesis. **(2) Intratumoral bacteria**: Within the liver, intratumoral bacteria (e.g., *E. faecalis* and *S. anginosus*) further shape an immunosuppressive tumor microenvironment characterized by increased MDSCs and reduced cytotoxic T-cell infiltration. **a. Bile acids**: Bile acids, such as DCA, induce SASP in hepatic stellate cells, while LCA modulates the Th17/Treg balance; **b. SCFAs**: SCFAs, including butyrate and acetate, regulate mucosal immune tolerance, hepatocyte proliferation, fibrosis, and ILC3 function. **c. Tryptophan derivatives**: tryptophan-derived metabolites, such as IAA and IPA, attenuate hepatocyte inflammation and support mucosal and hepatic immune homeostasis. SCFA, short-chain fatty acids; DCA, deoxycholic acid; LCA, lithocholic acid; SASP, senescence-associated secretory phenotypes; MDSCs, myeloid-derived suppressor cells; IAA, indole-3-acetic acid; IPA, indole-3-propionic acid. Figure created using BioRender (www.biorender.com).

promote intestinal homeostasis by supporting Treg differentiation, and in the case of butyrate, by maintaining mucosal integrity through anti-inflammatory, epigenetic, and anti-neoplastic mechanisms[66]. SCFAs act as histone deacetylase inhibitors and ligands for G protein-coupled receptors, such as GPR43, thereby modulating epigenetic and transcriptional programs in immune cells[67].

Recent work provided mechanistic insight by showing that acetate availability is markedly reduced in HCC-bearing mice due to loss of *Lactobacillus reuteri*, coinciding with heightened IL-17A production by ILC3s[68]. Restoration of acetate *via* fecal microbiota transplantation or direct supplementation suppresses tumor growth and enhances PD-1 blockade efficacy in HCC. Acetate exerts these effects by inhibiting histone deacetylase activity and promoting Sox13 acetylation to restrain IL-17A production in ILC3s, independent of GPR43 signaling. Together, these findings highlight SCFA acetate as a key microbial metabolite that shapes gut–liver immune crosstalk by modulating ILC3-driven inflammation in HCC. Butyrate promotes Treg differentiation and IL-10 production, reinforcing mucosal immune tolerance, while attenuating proinflammatory cytokine expression in macrophages and dendritic cells[66,69,70]. However, under conditions of microbial dysbiosis or bile acid depletion, excessive SCFA accumulation, particularly butyrate derived from inulin fermentation, can

paradoxically promote hepatocarcinogenesis by altering bile acid signaling and metabolic fluxes[71].

Tryptophan catabolites

Tryptophan-derived microbial metabolites constitute a major class of gut microbiota-dependent immunomodulators that shape intestinal and hepatic immune responses through cell type-specific signaling programs. A central mechanism underlying these effects is activation of the aryl hydrocarbon receptor (AhR), which integrates microbial signals to regulate cytokine production, immune barrier function, and inflammatory tone. Indole, for example, attenuates hepatic steatosis and inflammation by promoting PFKFB3 expression and suppressing pro-inflammatory macrophage activation through AhR signaling[72]. Indole-3-acetic acid (IAA) exerts broader immunoregulatory functions. IAA enhances IL-22 and REG3G expression in group 3 innate lymphoid cells (ILC3s) in the gut, thereby strengthening the mucosal immune barrier[73]. IAA mitigates inflammation and cytokine-driven lipogenesis in the liver by downregulating macrophage-derived cytokines and repressing FASN and SREBP-1c in hepatocytes through AhR-dependent pathways[74]. Similarly, indole-3-propionic acid (IPA) supports mucosal and hepatic immune homeostasis by upregulating epithelial tight junction proteins and dampening macrophage inflammatory responses. Together, these metabolite-specific actions underscore the nuanced and multifaceted ways in which gut microbiota-derived tryptophan metabolites fine-tune immune responses along the gut–liver axis. Additional microbial metabolites, such as N,N,N-trimethyl-5-aminovaleric acid, can exacerbate hepatic steatosis by inhibiting γ-butyrobetaine hydroxylase, reducing carnitine synthesis and impairing fatty acid oxidation[75].

Despite these advances, several mechanistic ambiguities remain regarding microbiome–immune interactions within the HCC microenvironment. Emerging evidence suggests that the immunologic effects of microbial metabolites are highly context-dependent, being shaped by disease etiology (viral, metabolic, or toxic), liver fibrosis stage, and host genetic variations in receptors, such as FXR, TGR5, and AhR. For example, bile acids may suppress or promote inflammation depending on the concentration and composition, while SCFAs can switch from antitumor-to-protumor functions under dysbiotic or nutrient-rich conditions. Furthermore, potential antagonism among microbial metabolites, such as bile acid-mediated repression of SCFA-producing bacteria or reciprocal inhibition between indole derivatives and secondary bile acids adds another layer of complexity to immune regulation. The origin of intratumoral microbiota is also unresolved. Whether these microbes arise from translocation of gut bacteria through a compromised gut–liver axis or through selective colonization within the immunometabolic niche of the tumor is still debated. It is plausible that gut-derived signals and intratumoral microbial metabolites act synergistically or competitively to modulate local immune states, influencing both immune exhaustion and therapeutic responsiveness. Understanding the crosstalk between microbial metabolism and host immunity offers promising avenues for therapeutic intervention in liver cancer through microbiota-targeted or metabolite-based modulation of immune pathways.

Intratumor microbiota and resident immunity

Beyond the well-recognized influence of the gut microbiota-derived metabolites, accumulating evidence highlights the existence of a distinct intratumor microbiota that exerts localized effects on immune regulation and tumor progression in HCC[76]. Recent studies have demonstrated that microbial DNA and viable bacteria can be detected within HCC nodules, suggesting that microbes not only translocate from the gut to the liver but may also establish persistent, functionally active communities within the TME[77]. By integrating metagenomic sequencing, spatial transcriptomics, and immune profiling across 242 tumor nodules and matched non-tumor liver tissues, it was revealed that each tumor nodule harbors a unique bacterial composition, reflecting profound intrahepatic microbial heterogeneity. The microbial signatures associated with intrahepatic metastasis (IM) *vs.* multicentric occurrence (MO) were further distinguished by two key pathologic subtypes of multifocal HCC with a diagnostic accuracy (AUC ≈ 0.80) based on a nine-bacterium panel. Notably, *Enterococcus faecalis* and *Streptococcus anginosus*, which are enriched in IM lesions, were functionally validated as active oncogenic modulators. Colonization by these species promote tumor invasion and metastasis by activating pro-inflammatory signaling pathways and fostering an immunosuppressive TME that is characterized by reduced cytotoxic T-cell infiltration and increased MDSC accumulation. Intratumoral bacteria, such as *E. faecalis* and *S. anginosus*, appear to influence resident hepatic immunity through direct and indirect routes. These intratumoral microorganisms interact closely with hepatic immune cells, shaping the local immune milieu and influencing antigen presentation. Furthermore, microbial colonization within the tumor may alter local oxygen gradients and metabolic signaling, particularly hypoxia-inducible and bile acid pathways, contributing to immune exhaustion and resistance to ICB therapy[78].

These findings collectively established intratumor bacteria as active participants in the evolution of the HCC immune microenvironment, rather than passive bystanders. The interplay between microbial presence and resident immune cell plasticity suggests that the hepatic microbiota, both gut-derived and intratumoral, represents an integral component of the "immune-microbial axis" in liver cancer. Understanding these complex relationships could elucidate the development of microbiome-based diagnostics to distinguish HCC subtypes, as well as targeted microbial modulation strategies to

restore antitumor immunity, and may improve immunotherapy efficacy.

Microbiome influence on HCC immunotherapy efficacy, side effects, and resistance

Accumulating evidence indicates that the gut microbiota is a key determinant of immunotherapy outcomes in HCC, influencing therapeutic efficacy, immune-related adverse events (irAEs), and the development of resistance[79-81]. Microbial composition and function shape systemic immunity and the TME through a close interaction with the gut–liver axis, underscoring the therapeutic potential of microbiome-targeted strategies in HCC (**Figure 4**).

Gut microbiota and enhanced immunotherapy efficacy

A diverse, functionally balanced microbial ecosystem is generally associated with more favorable immunotherapy outcomes, whereas dysbiosis promotes immune evasion, tumor progression, and reduced responsiveness to ICB[82-84]. Multi-strain *Enterobacteriaceae* formulations combined with PD-1/PD-L1 inhibitors have demonstrated synergistic antitumor effects[83]. The detrimental impact of broad-spectrum antibiotics further highlights the necessity of an intact microbiome for optimal ICB efficacy[85,86], which may involve remodeling the TME, enhancing $CD8^+$ T-cell activation and regulating T-cell differentiation programs.

Notably, bacterial regulation of the immune system is inseparable from bacterial metabolites in HCC. Owing to the

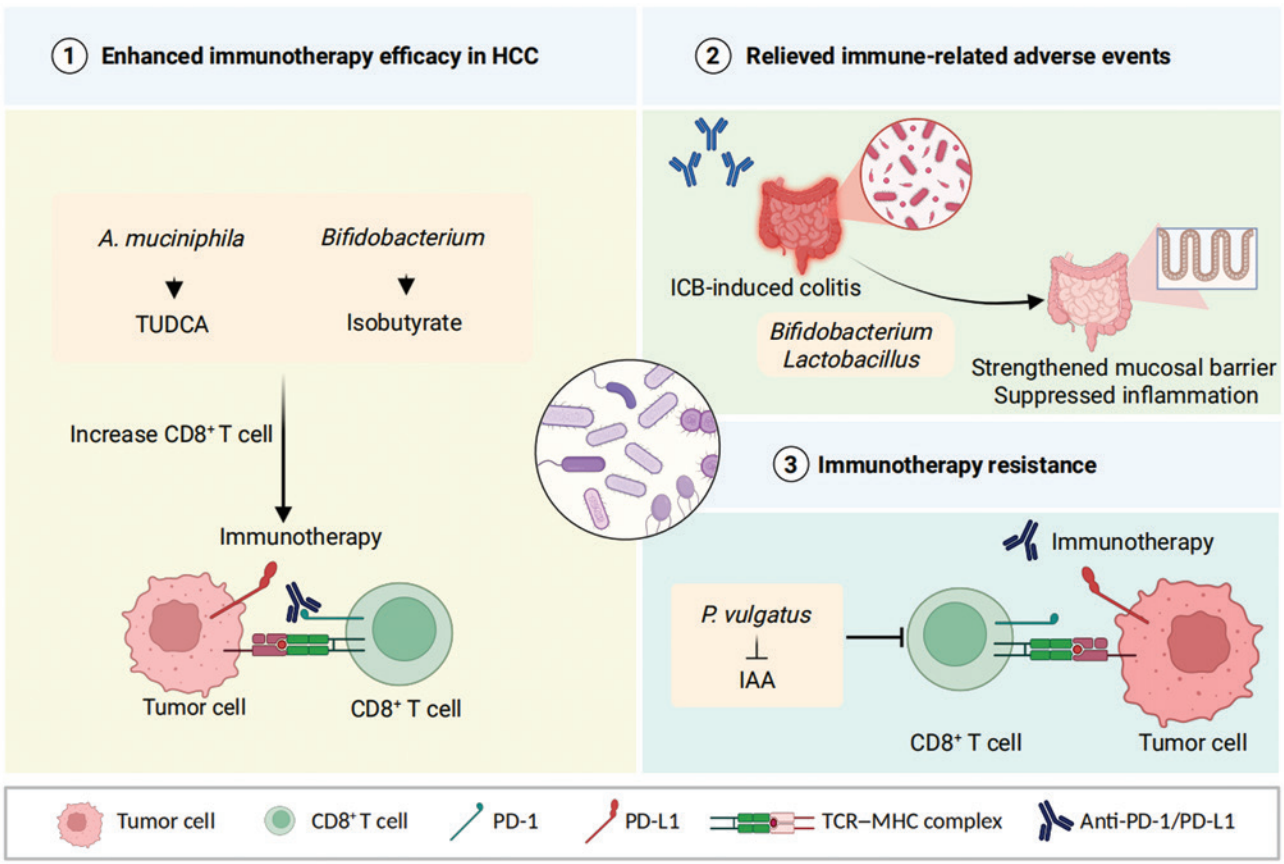


Figure 4 The impact of the microbiome on HCC immunotherapy: efficacy; immune-related adverse events, and resistance. **(1) Enhanced immunotherapy efficacy in HCC:** *A. muciniphila* and *Bifidobacterium*, together with the respective metabolites, TUDCA and isobutyrate, enhance the efficacy of immunotherapy by promoting the proliferation and functional activity of $CD8^+$ T cells. **(2) Relieved immune-related adverse events:** Beneficial commensal microbes, including *Bifidobacterium* and *Lactobacillus*, can strengthen the mucosal barrier and inhibit inflammatory responses, thereby alleviating irAEs. **(3) Immunotherapy resistance:** By contrast, some bacterial species can induce resistance to immunotherapy. For example, *P. vulgatus* impairs the cytotoxicity of $CD8^+$ T cells in the tumor microenvironment, which in turn suppresses the efficacy of immunotherapy. HCC, hepatocellular carcinoma; TUDCA, tauroursodeoxycholic acid; IAA, indole-3-acetic acid. Figure created using BioRender (www.biorender.com).

intestinal mucosal barrier function, most bacteria exert effects *via* the gut-liver axis, utilizing metabolic products, such as bile acids, SCFAs, and tryptophan, to modulate immune system function. Studies have shown that *Bifidobacterium* abundance is positively correlated with isobutyrate levels in the gut microbiota of healthy individuals. Both *Bifidobacterium* and isobutyrate can inhibit HCC growth by increasing $CD8^+$ T-cell counts and IFN-γ levels, while reducing JAK/STAT3 signaling in the TME[87]. Another study indicated that *Akkermansia muciniphila* promotes apoptosis of HCC cells and increases the proportion of $CD8^+$ T cells in the TME. Furthermore, *A. muciniphila* induces alterations in host bile acid metabolism, leading to a significant increase in serum tauroursodeoxycholic acid (TUDCA) levels, suggesting that *A. muciniphila* may enhance the efficacy of PD-1 monoclonal antibody immunotherapy against HCC through effects on bile acid metabolism[88]. Altered immune and microbial landscapes contribute to poor responses to monotherapy in MASLD-related HCC[89]. *A. muciniphila* alleviates hepatic steatosis by suppressing cholesterol biosynthesis and bile acid metabolism, while enhancing responsiveness to PD-1 blockade in MASLD-HCC through modulating MDSC activity and inhibiting M2 macrophage polarization[90]. In addition to bacteria-derived metabolites, a recent study demonstrated that inhibiting bile acid synthesis in hepatocytes enhances tumor-specific T-cell responses, reduces tumor growth, and sensitizes tumors to anti-PD-1 immunotherapy. In addition, different bile acid species exert distinct regulatory effects on $CD8^+$ T cells. Primary bile acids induce oxidative stress, whereas secondary bile acids suppress T-cell function *via* endoplasmic reticulum stress[91]. Collectively, these findings underscore the considerable potential of the gut microbiota and the metabolites as targets to enhance immunotherapy efficacy in HCC.

Clinical studies have shown the link between gut microbiota composition and ICB efficacy in HCC, expanding these insights beyond preclinical models. A study examined the dynamic changes in gut microbiota composition and characteristics in fecal samples from HCC patients undergoing anti-PD-1 immunotherapy following prior treatment with sorafenib. The findings revealed that responders exhibited greater microbial diversity in fecal microbiota compared to non-responders. Among the responder-enriched species, four *Lactobacillus* spp., *Bifidobacterium dentium*, and *Streptococcus thermophilus* were probiotic lactic acid bacteria that are beneficial to host metabolism and immunity by inhibiting the growth of pathogenic microorganisms, highlighting the potential of gut microbiota as a therapeutic target[92].

Microbiome contributions to irAEs

While ICB offers clinical benefit, irAEs significantly limit ICB use. The gut microbiota has a central role in irAE development with baseline microbial composition predicting susceptibility, especially for ICI-induced colitis[93,94]. Dysbiosis, whether spontaneous or antibiotic-induced, exacerbates irAEs by impairing epithelial barrier integrity, amplifying mucosal inflammation, and enhancing proinflammatory immune activation. Broad-spectrum antibiotic use not only worsens irAEs but also diminishes ICI efficacy, reflecting the dual role of the microbiome in regulating toxicity and therapeutic responsiveness[95,96].

Conversely, targeted microbiome modulation has shown promise in mitigating irAEs[97,98]. Beneficial microbes, such as *Bifidobacterium* and *Lactobacillus*, can reinforce the mucosal barrier, promote regulatory immune networks, and produce SCFAs that suppress inflammation[95]. Fecal microbiota transplantation (FMT) is currently being evaluated as a therapeutic option for refractory ICI-related colitis with early results demonstrating restoration of microbial diversity and improvement of clinical symptoms[99,100]. These findings highlight the potential of microbiome-based interventions to improve the safety profile of cancer immunotherapy.

Microbiome-driven immunotherapy resistance

The gut microbiota is increasingly recognized as a critical driver of immunotherapy resistance, which is a major barrier in HCC treatment[101,102]. Recent evidence has established a causal role for the gut microbiota in shaping immunotherapy resistance in HCC. Comparative analyses of responders and non-responders revealed marked microbial disparities. Specifically, *Phocaeicola vulgatus* was significantly enriched in non-responders and functioned as a key driver of anti-PD-1 resistance. FMT and single-strain colonization experiments confirmed that *P. vulgatus* suppresses immunotherapy efficacy, not by promoting tumor growth directly, but by impairing $CD8^+$ T cell cytotoxicity within the TME[84]. Mechanistically, *P. vulgatus* disrupts intestinal tryptophan metabolism by diverting IPA away from IAA synthesis due to the absence of IPDC and the presence of IOR, leading to systemic depletion of IAA. These findings identified *P. vulgatus* abundance and IAA deficiency as potential biomarkers of immunotherapy resistance and highlight the therapeutic promise of microbiota- and metabolite-targeted interventions to personalize and improve immunotherapy outcomes in HCC.

Antibiotic-induced disruption of microbiota composition further aggravates immunotherapy resistance, as demonstrated in clinical studies showing decreased survival among patients receiving antibiotics before or during ICB therapy[98]. As a result, microbiome preservation or deliberate modulation has emerged as an important strategy to overcome resistance. Notably, a phase II clinical trial (RENMIN-215) is currently evaluating the synergy of FMT with tislelizumab and fruquintinib to reverse anti-PD-1 resistance, exemplifying the translation of microbiome science into clinical intervention[103].

Therapeutic strategies of targeting the gut microbiome to enhance immunotherapy

Targeting the gut microbiome represents a novel frontier in enhancing the efficacy of immunotherapy for HCC. These strategies aim to restore a balanced microbial ecosystem, boost anti-tumor immunity, and mitigate irAEs[104] (**Figure 5**).

Probiotics and prebiotics

Probiotics are defined as live microorganisms that confer health benefits when administered. Common examples include well-studied bacterial genera, such as *Lactobacillus* and *Bifidobacterium*[105,106]. Emerging evidence supports the therapeutic potential of combining probiotics with ICB and anti-angiogenic agents in HCC[107]. Specific probiotic strains have demonstrated the ability to enhance treatment efficacy and mitigate irAEs. Supplementation with *Bifidobacterium* has been shown to significantly potentiate the anti-tumor effect of PD-1 blockade[87]. Similarly, *L. acidophilus* and *B. pseudolongum* improved intestinal barrier integrity and suppressed MASLD-HCC progression, suggesting the potential to enhance ICB efficacy[108,109]. Regarding toxicity management, specific probiotics effectively reduced the incidence of ICI-related colitis in mouse models. *Bifidobacterium* markedly alleviated colitis without compromising the anti-tumor activity of ICIs. This protective effect was abolished in Treg-deficient mice, indicating a Treg-dependent mechanism[110]. Collectively, these findings substantiated the strategic combination of probiotics with PD-1 inhibitors in HCC therapy.

Prebiotics, non-digestible substrates selectively utilized by beneficial microbes further support these immune-enhancing effects[111]. Various complex glycans, such as inulin, have been clinically evaluated as prebiotics[112]. A previous study demonstrated that the commensal bacterium, *Parabacteroides distasonis*, utilizes inulin to produce pentadecanoic acid, an odd-chain fatty acid, which in turn restores gut barrier function in MASH models. This restoration reduces serum lipopolysaccharide levels and hepatic pro-inflammatory cytokine expression, thereby suppressing disease progression[113]. Prebiotics also enhance the production of beneficial SCFAs, which subsequently stimulate systemic memory T-cell responses and promote T-cell infiltration and activation within the TME[114]. This mechanism is exemplified by artificial prebiotics, such as AHCC®, which has been shown to improve the efficacy of ICB by modifying gut microbiota composition and enhancing T-cell function in the TME[115].

Engineered bacterial strains

Engineered bacterial strains represent an advanced approach to precisely manipulate the gut–immune axis for therapeutic gain. Bacteria can be modified to produce targeted metabolites through microbial engineering, enhance immunostimulatory activity, or deliver therapeutic molecules directly to the gut or TME[116,117]. Although the application in HCC immunotherapy remains largely conceptual, the rationale is strong. Specific engineered strains could be designed to restore microbial functions lost in non-responders, promote antitumor immunity, or modulate signaling pathways essential for effective ICB. For example, a study revealed that the engineered *E. coli* Nissle 1917 strain enhanced the efficacy of PD-L1 blockade by boosting L-arginine-mediated T cell production and activation[118]. Such precision tools may ultimately overcome limitations of conventional probiotics and offer customizable solutions for microbiome-driven immunotherapy optimization.

FMT

FMT refers to the transfer of fecal microbiota from healthy donors to recipients, aiming to restore the diversity and functional capacity of the recipient gut microbiota. As a promising therapeutic strategy, FMT has demonstrated remarkable efficacy in replacing or modulating the existing gut microbiota of recipients, thereby exerting a positive regulatory effect on host physiologic status and disease outcomes with particularly prominent results in enhancing the responsiveness to immunotherapy in cancer treatment[119].

The effectiveness of FMT in regulating gut microbiota primarily lies in an ability to directly reshape the composition of gut microorganisms and restore the normal functions. A growing body of evidence indicates that FMT effectively restores the richness and diversity of gut microbiota in recipients by introducing a diverse microbial community from healthy donors[120]. The essence of this "ecosystem transplantation" is not targeting a single bacterial strain but introducing a complete, interacting microbial ecosystem, thereby re-establishing intestinal "colonization resistance." Colonization resistance refers to the inhibition of pathogenic microbial colonization and overgrowth through various mechanisms[121]. For example, antibiotic treatment disrupts gut microbiota diversity, in *Clostridioides difficile* infection (CDI), allowing *C. difficile* to proliferate. In contrast, FMT re-establishes colonization resistance by restoring a healthy microbial community, effectively treating recurrent CDI[122,123]. Studies have shown that the successful colonization of donor microbiota in the recipient' gut is crucial for achieving clinical remission in CDI patients undergoing FMT[124].

The potential mechanisms through which FMT replaces or modulates the existing gut microbiota to exert therapeutic effects are multifaceted. First, the transplanted healthy fecal microbiota can serve as a "functional donor pool," directly supplementing the recipient gut with a variety of beneficial microorganisms capable of colonizing the intestinal tract. These colonizing beneficial bacteria then compete with the recipient original harmful microorganisms for nutritional resources

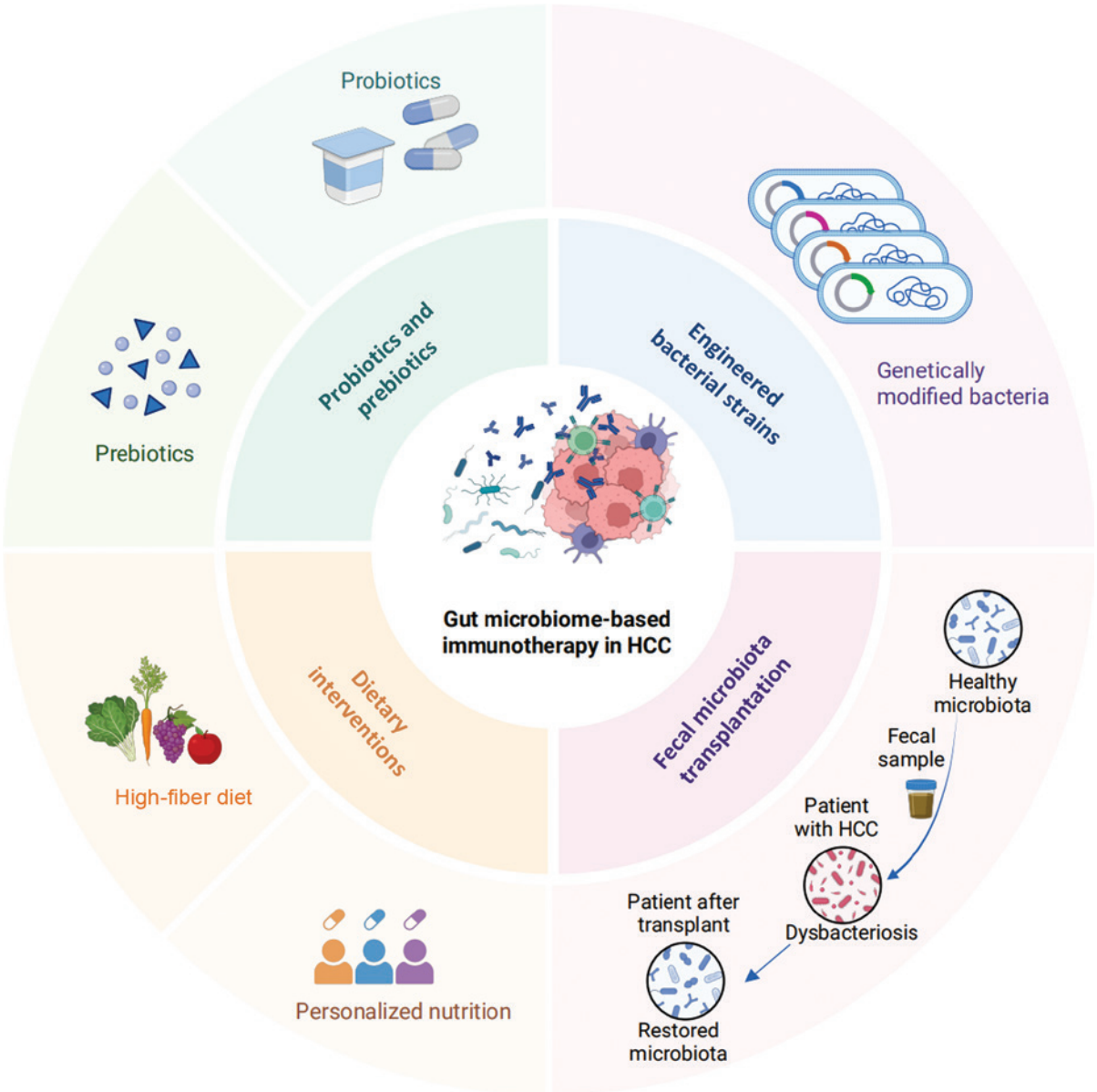


Figure 5 Microbiome-based therapeutic strategies to enhance immunotherapy efficacy in hepatocellular carcinoma. Conceptual overview of current and emerging microbiota-targeted interventions designed to optimize antitumor immunity in HCC. Strategies include probiotics and prebiotics, dietary interventions, engineered bacterial strains and fecal microbiota transplantation to restore a favorable microbial ecosystem in patients with dysbiosis. **Probiotics and prebiotics:** Probiotics (e.g., *Lactobacillus* and *Bifidobacterium*) and prebiotics (e.g., inulin) enhance the efficacy of immune checkpoint inhibitors and mitigate the toxicities in HCC. **Engineered bacteria strains:** Genetically modified bacterial strains represent a cutting-edge strategy to precisely modulate the gut-immune axis. **Fecal microbiota transplantation:** Fecal microbiota transplantation restores gut microbial diversity and function, showing promise in reversing immunotherapy resistance in HCC by restoring the gut microbiota in recipients by introducing a diverse microbial community from healthy donors. **Dietary interventions**: dietary interventions offer a non-invasive method to shape a favorable gut microbiome that can augment immunotherapy efficacy, highlighting the importance of personalized nutrition in HCC management. HCC, hepatocellular carcinoma. Figure created using BioRender (www.biorender.com).

and intestinal colonization sites, gradually replacing abnormal microbial populations and establishing a new, balanced microbial community[125]. Second, the transplanted microbiota can regulate the metabolic functions of the gut microbiota. FMT significantly increases the production of SCFAs[126]. Butyrate is not only the main energy source for colonic epithelial cells by helping to maintain the integrity of the intestinal barrier but also possesses potent immunomodulatory effects. For

example, butyrate can induce the production of Tregs, thereby inhibiting inflammatory responses and restoring immune tolerance[127]. In addition, FMT can regulate the gut microbiota by improving the intestinal microenvironment and immune homeostasis. Gut microbiota dysbiosis is often accompanied by mucosal inflammation[128] and FMT can effectively alleviate mucosal inflammation by re-establishing a healthy microbial community[129].

In addition, accumulating evidence suggests that the gut microbiota can regulate antitumor immune responses and influence the efficacy of cancer immunotherapy[130,131]. As an emerging adjuvant therapy for cancer, FMT has become a research hotspot for improving the efficacy and reducing the toxicity of ICIs by regulating the gut microbiota. For example, the FAB-HCC pilot study exemplifies this emerging translational research effort in HCC. The FAB-HCC pilot study is a phase II trial evaluating the efficacy of FMT combined with atezolizumab/bevacizumab in patients who have developed resistance to previous immunotherapy. This approach aims to overcome ICI resistance by reshaping the gut microbiota into a responder state, thereby re-sensitizing tumors to immunotherapy[132].

Dietary interventions

Dietary modification offers a practical, non-invasive means to modulate the gut microbiota and potentially enhance immunotherapy efficacy. Diet strongly influences microbial composition and metabolite production, including SCFAs and BAs. Among 128 melanoma patients receiving ICB therapy, higher dietary fiber intake was associated with significantly prolonged PFS with the most pronounced benefit observed in patients with adequate intake. This finding was corroborated in preclinical models, which demonstrated that a low-fiber diet impaired the response to anti-PD-1 therapy in mice[133]. The ketogenic diet elevated levels of the commensal bacterium, *Eisenbergiella massiliensis*, in a colorectal cancer mouse model, which showed a strong correlation with the principal ketone body, 3-hydroxybutyrate (3-HB). This cascade triggered a 3-HB-dependent T-cell antineoplastic response, boosting the efficacy of ICIs and elevating OS[134]. For the further development of such interventions, it is crucial to determine precisely how diet modulates microbial composition and augments antitumor immunity.

Although specific dietary guidelines for HCC patients undergoing immunotherapy remain emergent, the principle of fostering a diverse and health-promoting microbiome through personalized nutrition represents a promising adjunct strategy.

Ongoing clinical trials

Increasing recognition of the microbiome role in the immunotherapy response has spurred multiple clinical investigations. The FAB-HCC trial is actively evaluating the safety and efficacy of combining FMT with atezolizumab/bevacizumab in HCC patients who failed to sustain responses to first-line immunotherapy[132]. Beyond FMT-specific studies, broader clinical efforts aim to characterize microbial signatures predictive of immunotherapy outcomes, setting the stage for personalized microbiome-targeted interventions.

Collectively, these trials reflect a rapidly evolving landscape in which microbiome-based therapies, including probiotics, prebiotics, engineered strains, nutritional strategies, and FMT, are poised to complement and enhance immunotherapy for HCC (**Table 1**).

Conclusions and future perspectives

The gut microbiome has emerged as a pivotal regulator of the immunotherapy response in HCC, exerting multifaceted influences on antitumor immunity, therapeutic efficacy, and treatment-related toxicity. The microbiota actively shapes the hepatic immune landscape through the gut–liver axis, microbially derived metabolites, and even intratumoral microbial communities. Dysbiosis can impair immune surveillance, drive irAEs, and contribute to primary or acquired resistance, whereas a balanced microbial ecosystem can enhance effector T-cell function and improve responses to ICIs. This duality highlights the potential of the microbiome as a predictive biomarker and as a modifiable therapeutic target.

Probiotics and prebiotics

Although probiotics and prebiotics demonstrate promising potential in the treatment of HCC[81,135,136], several critical challenges remain, significantly hindering the clinical translation and widespread application.

First, the effects exhibit high strain specificity. The impact on gut microbiota modulation, immune function enhancement, and antitumor activity can vary substantially not only between different probiotic strains but even among subtypes of the same strain[137,138]. The optimal probiotic strain or combination for HCC patients has not been identified. Indiscriminate use may lead to suboptimal efficacy and could potentially exacerbate liver burden by disrupting intestinal microbial balance.

Second, there is a paucity of robust, HCC-specific clinical trial data. Current evidence largely derives from preclinical studies or small-scale clinical observations[139,140], which are insufficient to establish consistent, high-level evidence for clinical practice[141]. Moreover, existing research predominantly focuses on the short-term efficacy and acute adverse events of probiotics combined with ICB therapy[142]. Critical data regarding long-term safety, enduring antitumor effects, and OS benefits in HCC patients are lacking, undermining the foundation for standardized clinical protocols.

Table 1 Clinical trials initiated over the past 5 years exploring the role of the gut microbiome in HCC immunotherapy

Test drugs/regimen	Targets	Phase	Status	Estimated primary completion date	Trial identifier
Bifidobacterium bifidum oral product; carrilizumab; apatinib mesylate	PD-1; VEGFR-2	Phase I/II	Unknown	31-10-2024	NCT05620004
EXL01; PD-1 and CTLA-4 inhibitor	PD-1; CTLA-4	Phase II	Recruiting	12-12-2026	NCT06551272
FMT; atezolizumab; bevacizumab	PD-L1; VEGF-A	Phase II	Completed	17-03-2025	NCT05750030
FMT; atezolizumab; bevacizumab	PD-L1; VEGF-A	Phase II	Recruiting	03-2028	NCT05690048
CT-0508; pembrolizumab	HER2; PD-1	Phase 1	Active, not recruiting	31-12-2024	NCT04660929

Information retrieved from "https://clinicaltrials.gov/", as of 21 November 2025.
CAR-M, chimeric antigen receptor macrophage; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CT-0508, HER2-targeted CAR-macrophage cell therapy; EXL01, engineered microbial consortium; FMT, fecal microbiota transplantation; HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF-A, vascular endothelial growth factor A; VEGFR-2, vascular endothelial growth factor receptor 2.

Third, potential drug-drug interactions are poorly understood. HCC patients undergoing immunotherapy often receive concomitant medications, including targeted agents, chemotherapy, and hepatoprotective drugs[143]. Probiotics may interact with these therapies, yet the mechanisms and clinical significance of such interactions remain unexamined in systematic studies, posing a potential safety concern.

In conclusion, the application of probiotics and prebiotics in HCC immunotherapy remains exploratory. Advancing this field requires identifying the most effective, HCC-specific probiotic strains or combinations, conducting high-quality clinical trials to validate the efficacy and safety, and systematically investigating the interactions with established HCC therapeutics. These steps are essential to facilitate the standardized and personalized use of probiotics in HCC management, ultimately harnessing the full potential as synergistic adjunctive therapy.

Engineered bacteria

Engineered bacteria demonstrates significant potential in the field of HCC immunotherapy. By genetically engineering bacteria to perform targeted functions within the body, this approach offers a promising strategy to overcome the limitations associated with traditional probiotic applications[116]. However, this technology demands high precision in genetic modification and careful selection of suitable bacterial vectors[144]. A key challenge that remains to be addressed is achieving precise *in vivo* targeted delivery and long-term colonization of gene-edited bacteria in specific tissues or regions. Furthermore, the activity and proliferation of these engineered bacteria must be tightly regulated to prevent overgrowth, which could lead to systemic infection or adverse immune reactions. Existing control strategies, such as inducible promoter systems, require further optimization to adapt to the complex *in vivo* environment, ensuring effective activation when needed and reliable silencing when not[118,145].

Despite these challenges, engineered bacteria hold broad prospects in HCC immunotherapy. The precision of gene-editing technology in programming bacterial functions opens new possibilities for developing personalized treatment strategies for liver cancer. Engineered bacteria can counteract tumor drug resistance through various mechanisms, such as remodeling the tumor immune microenvironment, modulating tumor cell metabolism, or directly killing tumor cells. Beyond the therapeutic applications, these modified bacteria also have potential as novel tools for diagnosis and prognosis assessment[144].

In summary, engineered bacteria show transformative potential in HCC immunotherapy and provide patients with more effective and precise treatment strategies.

FMT

FMT has been demonstrated across various diseases in reshaping the existing gut microbiome. However, the clinical application still faces multiple challenges, particularly in the treatment of patients with HCC. Future research must focus on addressing these core issues to optimize the therapeutic efficacy and safety of FMT.

First, the safety of FMT remains a major concern, including infection risks and long-term stability. Due to the presence of diverse bacteria, viruses, fungi, and other microorganisms in stool, improper handling during preparation and transplantation may lead to pathogen transmission, resulting in sepsis or other infections[146]. Moreover, long-term safety data are still limited, and further evaluation is needed regarding the lasting effects of FMT on host physiology, immune function, and potential disease progression. For example, insufficient donor screening or suboptimal stool preparation could facilitate the transmission of pathogenic bacteria[146], which may cause severe infections and even fatal outcomes. To mitigate infection risks, rigorous

donor screening is essential, typically including criteria, such as age, general health, medical history, recent antibiotic use, and comprehensive laboratory testing. Second, the therapeutic effect of FMT largely depends on the quality and composition of the donor fecal microbiome[147]. High inter-donor variability in microbial communities is one of the main reasons for inconsistent FMT outcomes. Studies suggest that some "super-donors" may possess particularly beneficial microbial profiles that significantly enhance treatment efficacy. For example, donors with higher gut microbial diversity and an enrichment of specific beneficial taxa may offer greater therapeutic potential[148,149]. To address donor variability, future research should prioritize the identification of "super-donors" through advanced multi-omics analyses, including metagenomics and metabolomics, to identify biomarkers strongly correlated with positive treatment outcomes. Concurrently, the establishment of rigorously screened donor banks and the implementation of standardized protocols for stool processing and quality control are essential to ensure the consistency and therapeutic effectiveness of FMT products. Finally, current methods for FMT preparation, administration routes, and treatment frequency lack complete standardization, which limits broad clinical application and consistent efficacy evaluation[150,151].

These challenges collectively hinder the reproducibility and clinical translation of FMT in HCC therapy, underscoring the urgent need for targeted follow-up studies to overcome these bottlenecks. By addressing these key issues, FMT holds promise as a safe and effective adjunctive therapy for HCC, particularly in enhancing responses to immunotherapy.

In conclusion, the gut microbiome stands as a dynamic and influential regulator of HCC immunotherapy response. By diligently pursuing these future research directions, from precision profiling and next-generation therapeutics-to-personalized nutrition and artificial intelligence (AI) integration, we can unlock the full potential of microbiome-targeted interventions, ultimately transform the landscape of HCC treatment and significantly improve patient prognosis.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Jun Yu.
Wrote the paper: Mengrui Wu and Yating Zhang.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024; 74: 229-63.
2. Tan EY, Danpanichkul P, Yong JN, Yu Z, Tan DJH, Lim WH, et al. Liver cancer in 2021: global burden of disease study. J Hepatol. 2025; 82: 851-60.
3. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet. 2018; 391: 1163-73.
4. Ding J, Wen Z. Survival improvement and prognosis for hepatocellular carcinoma: analysis of the SEER database. BMC Cancer. 2021; 21: 1157.
5. Lau G, Obi S, Zhou J, Tateishi R, Qin S, Zhao H, et al. APASL clinical practice guidelines on systemic therapy for hepatocellular carcinoma-2024. Hepatol Int. 2024; 18: 1661-83.
6. Pail O, Lin MJ, Anagnostou T, Brown BD, Brody JD. Cancer vaccines and the future of immunotherapy. Lancet. 2025; 406: 189-202.
7. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med. 2020; 382: 1894-905.
8. Abou-Alfa GK, Lau G, Kudo M, Chan SL, Kelley RK, Furuse J, et al. Tremelimumab plus durvalumab in unresectable hepatocellular carcinoma. NEJM Evid. 2022; 1: EVIDoa2100070.
9. Galle PR, Decaens T, Kudo M, Qin S, Fonseca L, Sangro B, et al. Nivolumab (NIVO) plus ipilimumab (IPI) vs lenvatinib (LEN) or sorafenib (SOR) as first-line treatment for unresectable hepatocellular carcinoma (uHCC): first results from CheckMate 9DW. J Clin Oncol. 2024; 42: LBA4008.
10. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet. 2017; 389: 2492-502.
11. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol. 2018; 19: 940-52.
12. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, et al. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, phase III trial. J Clin Oncol. 2020; 38: 193-202.

13. Ganesan S, Mehnert J. Biomarkers for response to immune checkpoint blockade. Annu Rev Cancer Biol. 2020; 4: 331-51.
14. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013; 500: 415-21.
15. Totoki Y, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M,
et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. Nat Genet. 2014; 46: 1267-73.
16. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. N Engl J Med. 2017; 377: 2500-1.
17. Pinato DJ, Guerra N, Fessas P, Murphy R, Mineo T, Mauri FA, et al. Immune-based therapies for hepatocellular carcinoma. Oncogene. 2020; 39: 3620-37.
18. Xu LX, He MH, Dai ZH, Yu J, Wang JG, Li XC, et al. Genomic and transcriptional heterogeneity of multifocal hepatocellular carcinoma. Ann Oncol. 2019; 30: 990-7.
19. Dong LQ, Peng LH, Ma LJ, Liu DB, Zhang S, Luo SZ, et al. Heterogeneous immunogenomic features and distinct escape mechanisms in multifocal hepatocellular carcinoma. J Hepatol. 2020; 72: 896-908.
20. Schramm C. Bile acids, the microbiome, immunity, and liver tumors. N Engl J Med. 2018; 379: 888-90.
21. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010; 328: 228-31.
22. Mao K, Baptista AP, Tamoutounour S, Zhuang L, Bouladoux N, Martins AJ, et al. Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. Nature. 2018; 554: 255-9.
23. Shetty S, Lalor PF, Adams DH. Liver sinusoidal endothelial cells — gatekeepers of hepatic immunity. Nat Rev Gastroenterol Hepatol. 2018; 15: 555-67.
24. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, et al. Gut microbiome–mediated bile acid metabolism regulates liver cancer via NKT cells. Science. 2018; 360: eaan5931.
25. Tao H, Chen X. Role of CXCR6-deficient natural killer T cells and CD4 T cells in hepatocarcinogenesis. Gastroenterology. 2019; 157: 1169-70.
26. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol. 2009; 9: 799-809.
27. de Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. Gut. 2022; 71: 1020-32.
28. Yang X, Lu D, Zhuo J, Lin Z, Yang M, Xu X. The gut-liver axis in immune remodeling: new insight into liver diseases. Int J Biol Sci. 2020; 16: 2357-66.
29. De Schepper S, Verheijden S, Aguilera-Lizarraga J, Viola MF, Boesmans W, Stakenborg N, et al. Self-maintaining gut macrophages are essential for intestinal homeostasis. Cell. 2018; 175: 400-15.e13.
30. Honda M, Surewaard BGJ, Watanabe M, Hedrick CC, Lee WY, Brown K, et al. Perivascular localization of macrophages in the intestinal mucosa is regulated by Nr4a1 and the microbiome. Nat Commun. 2020; 11: 1329.
31. Chiang HY, Lu HH, Sudhakar JN, Chen YW, Shih NS, Weng YT, et al. IL-22 initiates an IL-18-dependent epithelial response circuit to enforce intestinal host defence. Nat Commun. 2022; 13: 874.
32. Guilliams M, Bonnardel J, Haest B, Vanderborght B, Wagner C, Remmerie A, et al. Spatial proteogenomics reveals distinct and evolutionarily conserved hepatic macrophage niches. Cell. 2022; 185: 379-96.e38.
33. Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. Cell Metab. 2016; 24: 41-50.
34. Leshem A, Liwinski T, Elinav E. Immune-microbiota interplay and colonization resistance in infection. Mol Cell. 2020; 78: 597-613.
35. Bode C, Kugler V, Bode JC. Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. J Hepatol. 1987; 4: 8-14.
36. Tilg H, Moschen AR, Kaneider NC. Pathways of liver injury in alcoholic liver disease. J Hepatol. 2011; 55: 1159-61.
37. Chen P, Stärkel P, Turner JR, Ho SB, Schnabl B. Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor I and mediates alcoholic liver disease in mice. Hepatology. 2015; 61: 883-94.
38. Fairfield B, Schnabl B. Gut dysbiosis as a driver in alcohol-induced liver injury. JHEP Rep. 2021; 3: 100220.
39. Grander C, Adolph TE, Wieser V, Lowe P, Wrzosek L, Gyongyosi B, et al. Recovery of ethanol-induced *Akkermansia muciniphila* depletion ameliorates alcoholic liver disease. Gut. 2018; 67: 891-901.
40. Duan Y, Llorente C, Lang S, Brandl K, Chu H, Jiang L, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature. 2019; 575: 505-11.
41. Duan Y, Chu H, Brandl K, Jiang L, Zeng S, Meshgin N, et al. CRIg on liver macrophages clears pathobionts and protects against alcoholic liver disease. Nat Commun. 2021; 12: 7172.
42. Luther J, Garber JJ, Khalili H, Dave M, Bale SS, Jindal R, et al. Hepatic injury in nonalcoholic steatohepatitis contributes to altered intestinal permeability. Cell Mol Gastroenterol Hepatol. 2015; 1: 222-32.
43. Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology. 2013; 57: 601-9.
44. Xie C, Yagai T, Luo Y, Liang X, Chen T, Wang Q, et al. Activation of intestinal hypoxia-inducible factor 2α during obesity contributes to hepatic steatosis. Nat Med. 2017; 23: 1298-308.
45. Tilg H, Adolph TE, Dudek M, Knolle P. Non-alcoholic fatty liver disease: the interplay between metabolism, microbes and immunity. Nat Metab. 2021; 3: 1596-607.
46. Johnson EL, Heaver SL, Waters JL, Kim BI, Bretin A, Goodman AL, et al. Sphingolipids produced by gut bacteria enter host metabolic pathways impacting ceramide levels. Nat Commun. 2020; 11: 2471.
47. Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, et al. Gut microbiome-based metagenomic signature for non-invasive

detection of advanced fibrosis in human nonalcoholic fatty liver disease. Cell Metab. 2017; 25: 1054-62.e5.
48. Alferink LJM, Radjabzadeh D, Erler NS, Vojinovic D, Medina-Gomez C, Uitterlinden AG, et al. Microbiomics, metabolomics, predicted metagenomics, and hepatic steatosis in a population-based study of 1,355 adults. Hepatology. 2021; 73: 968-82.
49. Frost F, Kacprowski T, Rühlemann M, Pietzner M, Bang C, Franke A, et al. Long-term instability of the intestinal microbiome is associated with metabolic liver disease, low microbiota diversity, diabetes mellitus and impaired exocrine pancreatic function. Gut. 2021; 70: 522-30.
50. Riva A, Gray EH, Azarian S, Zamalloa A, McPhail MJW, Vincent RP, et al. Faecal cytokine profiling as a marker of intestinal inflammation in acutely decompensated cirrhosis. JHEP Rep. 2020; 2: 100151.
51. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature. 2014; 513: 59-64.
52. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-β signaling and hepatic fibrosis. Nat Med. 2007; 13: 1324-32.
53. Komiyama S, Yamada T, Takemura N, Kokudo N, Hase K, Kawamura YI. Profiling of tumour-associated microbiota in human hepatocellular carcinoma. Sci Rep. 2021; 11: 10589.
54. Liu B, Zhou Z, Jin Y, Lu J, Feng D, Peng R, et al. Hepatic stellate cell activation and senescence induced by intrahepatic microbiota disturbances drive progression of liver cirrhosis toward hepatocellular carcinoma. J Immunother Cancer. 2022; 10: e003069.
55. Ponziani FR, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. Hepatology. 2019; 69: 107-20.
56. Liu Q, Li F, Zhuang Y, Xu J, Wang J, Mao X, et al. Alteration in gut microbiota associated with hepatitis B and non-hepatitis virus related hepatocellular carcinoma. Gut Pathog. 2019; 11: 1.
57. Wang X, Fang Y, Liang W, Cai Y, Wong CC, Wang J, et al. Gut–liver translocation of pathogen *Klebsiella pneumoniae* promotes hepatocellular carcinoma in mice. Nat Microbiol. 2025; 10: 169-84.
58. Zhang Y, Liu W, Wong CC, Song Q, Zhang X, Zhou Q, et al. *Catenibacterium mitsuokai* promotes hepatocellular carcinogenesis by binding to hepatocytes and generating quinolinic acid. Cell Metab. 2025; 37: 1998-2013.e7.
59. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013; 499: 97-101.
60. Hang S, Paik D, Yao L, Kim E, Trinath J, Lu J, et al. Bile acid metabolites control T_H17 and T_{reg} cell differentiation. Nature. 2019; 576: 143-8.
61. Song X, Sun X, Oh SF, Wu M, Zhang Y, Zheng W, et al. Microbial bile acid metabolites modulate gut RORγ^+ regulatory T cell homeostasis. Nature. 2020; 577: 410-5.
62. Loo TM, Kamachi F, Watanabe Y, Yoshimoto S, Kanda H, Arai Y, et al. Gut Microbiota promotes obesity-associated liver cancer through PGE_2-mediated suppression of antitumor immunity. Cancer Discov. 2017; 7: 522-38.
63. Zheng C, Wang L, Zou T, Lian S, Luo J, Lu Y, et al. Ileitis promotes MASLD progression via bile acid modulation and enhanced TGR5 signaling in ileal $CD8^+$ T cells. J Hepatol. 2024; 80: 764-77.
64. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. J Immunol. 2009; 183: 6251-61.
65. Pols TWH, Nomura M, Harach T, Lo Sasso G, Oosterveer MH, Thomas C, et al. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. Cell Metab. 2011; 14: 747-57.
66. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504: 446-50.
67. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell. 2016; 165: 1332-45.
68. Hu C, Xu B, Wang X, Wan WH, Lu J, Kong D, et al. Gut microbiota-derived short-chain fatty acids regulate group 3 innate lymphoid cells in HCC. Hepatology. 2023; 77: 48-64.
69. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc Natl Acad Sci U S A. 2014; 111: 2247-52.
70. Andrusaite A, Lewis J, Frede A, Farthing A, Kästele V, Montgomery J, et al. Microbiota-derived butyrate inhibits cDC development via HDAC inhibition, diminishing their ability to prime T cells. Mucosal Immunol. 2024; 17: 1199-211.
71. Singh V, Yeoh BS, Chassaing B, Xiao X, Saha P, Aguilera Olvera R, et al. Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer. Cell. 2018; 175: 679-94.e22.
72. Ma L, Li H, Hu J, Zheng J, Zhou J, Botchlett R, et al. Indole alleviates diet-induced hepatic steatosis and inflammation in a manner involving myeloid cell 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3. Hepatology. 2020; 72: 1191-203.
73. Hendrikx T, Duan Y, Wang Y, Oh JH, Alexander LM, Huang W, et al. Bacteria engineered to produce IL-22 in intestine induce expression of REG3G to reduce ethanol-induced liver disease in mice. Gut. 2019; 68: 1504-15.
74. Krishnan S, Ding Y, Saedi N, Choi M, Sridharan GV, Sherr DH, et al. Gut microbiota-derived tryptophan metabolites modulate inflammatory response in hepatocytes and macrophages. Cell Rep. 2018; 23: 1099-111.
75. Zhao M, Zhao L, Xiong X, He Y, Huang W, Liu Z, et al. TMAVA, a metabolite of intestinal microbes, is increased in plasma from patients with liver steatosis, inhibits γ-butyrobetaine hydroxylase, and exacerbates fatty liver in mice. Gastroenterology. 2020; 158: 2266-81.e27.
76. Yang L, Li A, Wang Y, Zhang Y. Intratumoral microbiota: roles in cancer initiation, development and therapeutic efficacy. Signal Transduct Target Ther. 2023; 8: 35.

77. Lu Y, Xu L, Chen W, Liu W, Zhang Y, Zhou Q, et al. Intrahepatic microbial heterogeneity in multifocal hepatocellular carcinoma and its association with host genomic and transcriptomic alterations. Cancer Discov. 2025; 15: 1630-48.
78. Luo YC, Huang XT, Wang R, Lin YJ, Sun JX, Li KF, et al. Advancements in understanding tumor-resident bacteria and their application in cancer therapy. Mil Med Res. 2025; 12: 38.
79. Gao YQ, Tan YJ, Fang JY. Roles of the gut microbiota in immune-related adverse events: mechanisms and therapeutic intervention. Nat Rev Clin Oncol. 2025; 22: 499-516.
80. Du J, Guan Y, Zhang E. Regulatory role of gut microbiota in immunotherapy of hepatocellular carcinoma. Hepatol Int. 2025; 19: 507-18.
81. Huang M, Ji Q, Huang H, Wang X, Wang L. Gut microbiota in hepatocellular carcinoma immunotherapy: immune microenvironment remodeling and gut microbiota modification. Gut Microbes. 2025; 17: 2486519.
82. Luo W, Guo S, Zhou Y, Zhao J, Wang M, Sang L, et al. Hepatocellular carcinoma: how the gut microbiota contributes to pathogenesis, diagnosis, and therapy. Front Microbiol. 2022; 13: 873160.
83. Wu D, Fan Y, Zhang M, Wang X, He X, Guo X, et al. Tumor-suppressing multi-enterobacteria and PD-1/PD-L1 immune checkpoint inhibitor combination improves the outcome of hepatocellular carcinoma therapy. Front Immunol. 2025; 16: 1598436.
84. Zhao CN, Li SS, Yau T, Chen WQ, Ji R, Guan XY, et al. *Phocaeicola vulgatus* induces immunotherapy resistance in hepatocellular carcinoma via reducing indoleacetic acid production. Cell Rep Med. 2025; 6: 102370.
85. Cheung KS, Lam LK, Seto WK, Leung WK. Use of antibiotics during immune checkpoint inhibitor treatment is associated with lower survival in hepatocellular carcinoma. Liver Cancer. 2021; 10: 606-14.
86. Fessas P, Naeem M, Pinter M, Marron TU, Szafron D, Balcar L, et al. Early antibiotic exposure is not detrimental to therapeutic effect from immunotherapy in hepatocellular carcinoma. Liver Cancer. 2021; 10: 583-92.
87. Huo R, Xu QG, You YQ, Chen YL, Su GJ, Yang KR, et al. *Bifidobacterium* boosts anti-PD-1 effectiveness through JAK pathway in hepatocellular carcinoma. npj Precis Oncol. 2025; 9: 251.
88. Lan X, Ma J, Huang Z, Xu Y, Hu Y. *Akkermansia muciniphila* might improve anti-PD-1 therapy against HCC by changing host bile acid metabolism. J Gene Med. 2024; 26: e3639.
89. Pfister D, Núñez NG, Pinyol R, Govaere O, Pinter M, Szydlowska M, et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature. 2021; 592: 450-6.
90. Wu XQ, Ying F, Chung KPS, Leung CON, Leung RWH, So KKH, et al. Intestinal *Akkermansia muciniphila* complements the efficacy of PD1 therapy in MAFLD-related hepatocellular carcinoma. Cell Rep Med. 2025; 6: 101900.
91. Varanasi SK, Chen D, Liu Y, Johnson MA, Miller CM, Ganguly S, et al. Bile acid synthesis impedes tumor-specific T cell responses during liver cancer. Science. 2025; 387: 192-201.
92. Zheng Y, Wang T, Tu X, Huang Y, Zhang H, Tan D, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. J Immunother Cancer. 2019; 7: 193.
93. Seton-Rogers S. Microbiota links to immunotherapy toxicity. Nat Rev Cancer. 2021; 21: 540.
94. Zhang Y, Cheng S, Zou H, Han Z, Xie T, Zhang B, et al. Correlation of the gut microbiome and immune-related adverse events in gastrointestinal cancer patients treated with immune checkpoint inhibitors. Front Cell Infect Microbiol. 2023; 13: 1099063.
95. Tan B, Liu YX, Tang H, Chen D, Xu Y, Chen MJ, et al. Gut microbiota shed new light on the management of immune-related adverse events. Thorac Cancer. 2022; 13: 2681-91.
96. Chang ALS, Zaba L, Kwong BY. Immunotherapy for keratinocyte cancers. Part II: identification and management of cutaneous side effects of immunotherapy treatments. J Am Acad Dermatol. 2023; 88: 1243-55.
97. Aarnoutse R, Ziemons J, Penders J, Rensen SS, de Vos-Geelen J, Smidt ML. The clinical link between human intestinal microbiota and systemic cancer therapy. Int J Mol Sci. 2019; 20: 4145.
98. Derosa L, Zitvogel L. Antibiotics impair immunotherapy for urothelial cancer. Nat Rev Urol. 2020; 17: 605-6.
99. Park R, Umar S, Kasi A. Immunotherapy in colorectal cancer: potential of fecal transplant and microbiota-augmented clinical trials. Curr Colorectal Cancer Rep. 2020; 16: 81-8.
100. Ciernikova S, Sevcikova A, Drgona L, Mego M. Modulating the gut microbiota by probiotics, prebiotics, postbiotics, and fecal microbiota transplantation: an emerging trend in cancer patient care. Biochim Biophys Acta Rev Cancer. 2023; 1878: 188990.
101. Wang J, Zhu N, Su X, Yang R. Gut microbiota: a double-edged sword in immune checkpoint blockade immunotherapy against tumors. Cancer Lett. 2024; 582: 216582.
102. Chen L, Li B, Zu M, Reis RL, Kundu SC, Xiao B. Advances and mechanisms of gut microbiota modulation in enhancing immune checkpoint inhibitor efficacy. Semin Cancer Biol. 2025; 114: 150-72.
103. Zhao W, Lei J, Ke S, Chen Y, Xiao J, Tang Z, et al. Fecal microbiota transplantation plus tislelizumab and fruquintinib in refractory microsatellite stable metastatic colorectal cancer: an open-label, single-arm, phase II trial (RENMIN-215). EClinicalMedicine. 2023; 66: 102315.
104. Fernandes MR, Aggarwal P, Costa RGF, Cole AM, Trinchieri G. Targeting the gut microbiota for cancer therapy. Nat Rev Cancer. 2022; 22: 703-22.
105. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014; 11: 506-14.
106. Badgeley A, Anwar H, Modi K, Murphy P, Lakshmikuttyamma A. Effect of probiotics and gut microbiota on anti-cancer drugs: mechanistic perspectives. Biochim Biophys Acta Rev Cancer. 2021; 1875: 188494.

107. Wang Y, Wu Z, Zhu X, Zheng Y, Yang Y, Tu J, et al. Concomitant medications alter clinical outcomes in patients with advanced digestive tract cancer receiving PD-1 checkpoint inhibitors combined with antiangiogenetic agents. J Gastrointest Cancer. 2024; 55: 1388-400.
108. Song Q, Zhang X, Liu W, Wei H, Liang W, Zhou Y, et al. *Bifidobacterium pseudolongum*-generated acetate suppresses non-alcoholic fatty liver disease-associated hepatocellular carcinoma. J Hepatol. 2023; 79: 1352-65.
109. Lau HCH, Zhang X, Ji F, Lin Y, Liang W, Li Q, et al. *Lactobacillus acidophilus* suppresses non-alcoholic fatty liver disease-associated hepatocellular carcinoma through producing valeric acid. EBioMedicine. 2024; 100: 104952.
110. Wang F, Yin Q, Chen L, Davis MM. *Bifidobacterium* can mitigate intestinal immunopathology in the context of CTLA-4 blockade. Proc Natl Acad Sci U S A. 2018; 115: 157-61.
111. Monteagudo-Mera A, Rastall RA, Gibson GR, Charalampopoulos D, Chatzifragkou A. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Appl Microbiol Biotechnol. 2019; 103: 6463-72.
112. Chambers ES, Byrne CS, Morrison DJ, Murphy KG, Preston T, Tedford C, et al. Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial. Gut. 2019; 68: 1430-8.
113. Wei W, Wong CC, Jia Z, Liu W, Liu C, Ji F, et al. *Parabacteroides distasonis* uses dietary inulin to suppress NASH via its metabolite pentadecanoic acid. Nat Microbiol. 2023; 8: 1534-48.
114. Liu X, Wang L, Jing N, Jiang G, Liu Z. Biostimulating gut microbiome with bilberry anthocyanin combo to enhance anti-PD-L1 efficiency against murine colon cancer. Microorganisms. 2020; 8: 175.
115. Park HJ, Boo S, Park I, Shin MS, Takahashi T, Takanari J, et al. AHCC®, a standardized extract of cultured *Lentinula edodes* mycelia, promotes the anti-tumor effect of dual immune checkpoint blockade effect in murine colon cancer. Front Immunol. 2022; 13: 875872.
116. Gurbatri CR, Arpaia N, Danino T. Engineering bacteria as interactive cancer therapies. Science. 2022; 378: 858-64.
117. Jin WB, Li TT, Huo D, Qu S, Li XV, Arifuzzaman M, et al. Genetic manipulation of gut microbes enables single-gene interrogation in a complex microbiome. Cell. 2022; 185: 547-62.e22.
118. Canale FP, Basso C, Antonini G, Perotti M, Li N, Sokolovska A, et al. Metabolic modulation of tumours with engineered bacteria for immunotherapy. Nature. 2021; 598: 662-6.
119. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science. 2021; 371: 602-9.
120. Kelly CR, Yen EF, Grinspan AM, Kahn SA, Atreja A, Lewis JD, et al. Fecal microbiota transplantation is highly effective in real-world practice: initial results from the FMT National Registry. Gastroenterology. 2021; 160: 183-92.e3.
121. Caballero-Flores G, Pickard JM, Núñez G. Microbiota-mediated colonization resistance: mechanisms and regulation. Nat Rev Microbiol. 2023; 21: 347-60.
122. Yadegar A, Pakpoor S, Ibrahim FF, Nabavi-Rad A, Cook L, Walter J, et al. Beneficial effects of fecal microbiota transplantation in recurrent *Clostridioides difficile* infection. Cell Host Microbe. 2023; 31: 695-711.
123. Zuo T, Wong SH, Cheung CP, Lam K, Lui R, Cheung K, et al. Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in *Clostridium difficile* infection. Nat Commun. 2018; 9: 3663.
124. Ianiro G, Punčochář M, Karcher N, Porcari S, Armanini F, Asnicar F, et al. Variability of strain engraftment and predictability of microbiome composition after fecal microbiota transplantation across different diseases. Nat Med. 2022; 28: 1913-23.
125. Onifade IA, Sunbare-Funto OJ, Mbah CE, Ajibade OA, Oyawoye OM, Aborode AT, et al. Faecal microbial transplant. Adv Biomark Sci Technol. 2024; 6: 20-34.
126. Lou X, Xue J, Shao R, Yang Y, Ning D, Mo C, et al. Fecal microbiota transplantation and short-chain fatty acids reduce sepsis mortality by remodeling antibiotic-induced gut microbiota disturbances. Front Immunol. 2022; 13: 1063543.
127. He L, Zhong Z, Wen S, Li P, Jiang Q, Liu F. Gut microbiota-derived butyrate restores impaired regulatory T cells in patients with AChR myasthenia gravis via mTOR-mediated autophagy. Cell Commun Signal. 2024; 22: 215.
128. Shi N, Li N, Duan X, Niu H. Interaction between the gut microbiome and mucosal immune system. Mil Med Res. 2017; 4: 14.
129. Jeon SR, Chai J, Kim C, Lee CH. Current evidence for the management of inflammatory bowel diseases using fecal microbiota transplantation. Curr Infect Dis Rep. 2018; 20: 21.
130. Yang Y, An Y, Dong Y, Chu Q, Wei J, Wang B, et al. Fecal microbiota transplantation: no longer cinderella in tumour immunotherapy. EBioMedicine. 2024; 100: 104967.
131. Liu P, Liu Z, Wang J, Wang J, Gao M, Zhang Y, et al. Immunoregulatory role of the gut microbiota in inflammatory depression. Nat Commun. 2024; 15: 3003.
132. Pomej K, Frick A, Scheiner B, Balcar L, Pajancic L, Klotz A, et al. Study protocol: fecal microbiota transplant combined with atezolizumab/bevacizumab in patients with hepatocellular carcinoma who failed to achieve or maintain objective response to atezolizumab/bevacizumab – the FAB-HCC pilot study. PLoS One. 2025; 20: e0321189.
133. Spencer CN, McQuade JL, Gopalakrishnan V, McCulloch JA, Vetizou M, Cogdill AP, et al. Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. Science. 2021; 374: 1632-40.
134. Ferrere G, Tidjani Alou M, Liu P, Goubet AG, Fidelle M, Kepp O, et al. Ketogenic diet and ketone bodies enhance the anticancer effects of PD-1 blockade. JCI Insight. 2021; 6: e145207.
135. Wan MLY, El-Nezami H. Targeting gut microbiota in hepatocellular carcinoma: probiotics as a novel therapy. Hepatobiliary Surg Nutr. 2018; 7: 11-20.

136. Yu J, Chen X, Yang X, Zhang B. Understanding gut dysbiosis for hepatocellular carcinoma diagnosis and treatment. Trends Endocrinol Metab. 2024; 35: 1006-20.
137. Jiang SS, Xie YL, Xiao XY, Kang ZR, Lin XL, Zhang L, et al. *Fusobacterium nucleatum*-derived succinic acid induces tumor resistance to immunotherapy in colorectal cancer. Cell Host Microbe. 2023; 31: 781-97.e9.
138. Wang X, Fang Y, Liang W, Wong CC, Qin H, Gao Y, et al. Fusobacterium nucleatum facilitates anti-PD-1 therapy in microsatellite stable colorectal cancer. Cancer Cell. 2024; 42: 1729-46.e8.
139. Li J, Sung CYJ, Lee N, Ni Y, Pihlajamäki J, Panagiotou G, et al. Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. Proc Natl Acad Sci U S A. 2016; 113: E1306-15.
140. Xu J, Zhan Q, Fan Y, Lo EKK, Zhang F, Yu Y, et al. Clinical aspects of gut microbiota in hepatocellular carcinoma management. Pathogens. 2021; 10: 782.
141. Ren S, Zhang Y, Wang X, Su J, Wang X, Yuan Z, et al. Emerging insights into the gut microbiota as a key regulator of immunity and response to immunotherapy in hepatocellular carcinoma. Front Immunol. 2025; 16: 1526967.
142. Shi K, Zhang Q, Zhang Y, Bi Y, Zeng X, Wang X. Association between probiotic therapy and the risk of hepatocellular carcinoma in patients with hepatitis B-related cirrhosis. Front Cell Infect Microbiol. 2023; 12: 1104399.
143. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on the management of hepatocellular carcinoma. J Hepatol. 2025; 82: 315-74.
144. Aggarwal N, Kitano S, Puah GRY, Kittelmann S, Hwang IY, Chang MW. Microbiome and human health: current understanding, engineering, and enabling technologies. Chem Rev. 2023; 123: 31-72.
145. Chappell TC, Nair NU. Engineered lactobacilli display anti-biofilm and growth suppressing activities against *Pseudomonas aeruginosa*. npj Biofilms Microbiomes. 2020; 6: 48.
146. Park SY, Seo GS. Fecal microbiota transplantation: is it safe? Clin Endosc. 2021; 54: 157-60.
147. Bibbò S, Settanni CR, Porcari S, Bocchino E, Ianiro G, Cammarota G, et al. Fecal microbiota transplantation: screening and selection to choose the optimal donor. J Clin Med. 2020; 9: 1757.
148. Rondinella D, Quaranta G, Rozera T, Dargenio P, Fancello G, Venturini I, et al. Donor screening for fecal microbiota transplantation with a direct stool testing-based strategy: a prospective cohort study. Microbes Infect. 2024; 26: 105341.
149. Wilson BC, Vatanen T, Cutfield WS, O'Sullivan JM. The super-donor phenomenon in fecal microbiota transplantation. Front Cell Infect Microbiol. 2019; 9: 2.
150. Kim KO, Gluck M. Fecal microbiota transplantation: an update on clinical practice. Clin Endosc. 2019; 52: 137-43.
151. Peery AF, Kelly CR, Kao D, Vaughn BP, Lebwohl B, Singh S, et al. AGA clinical practice guideline on fecal microbiota-based therapies for select gastrointestinal diseases. Gastroenterology. 2024; 166: 409-34.

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