Dai *et al. Hepatoma Res* 2023;9:39
DOI: 10.20517/2394-5079.2023.33

Hepatoma Research

Review

Open Access

Check for updates

The role of gut microbiome and fecal microbiota transplantation in liver cancer and related complications: mechanisms and therapeutic potentials

Min Dai[1], Rashid N. Lui[1,2,3], Louis H. S. Lau[1,2,3]

[1]Division of Gastroenterology and Hepatology, Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China.
[2]Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong, China.
[3]State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China.

Correspondence to: Prof. Louis H.S. Lau, Division of Gastroenterology and Hepatology, Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, 9/F, Lui Che Woo Clinical Sciences Building, Prince of Wales Hospital, Shatin, Hong Kong, China. E-mail: louishslau@cuhk.edu.hk

How to cite this article: Dai M, Lui RN, Lau LHS. The role of gut microbiome and fecal microbiota transplantation in liver cancer and related complications: mechanisms and therapeutic potentials. *Hepatoma Res* 2023;9:39. https://dx.doi.org/10.20517/2394-5079.2023.33

Received: 15 Apr 2023 **First Decision:** 15 Jun 2023 **Revised:** 18 Jul 2023 **Accepted:** 7 Aug 2023 **Published:** 10 Aug 2023

Academic Editor: Amedeo Lonardo **Copy Editor:** Dan Zhang **Production Editor:** Dan Zhang

Abstract

Liver cancer is the sixth commonest cancer and the third leading cause of cancer mortality worldwide. Accumulating evidence suggests a pivotal role of the gut microbiome in the progression of chronic liver disease and the subsequent development of liver cancer. Additionally, gut microbiome has been shown to contribute to the hosts' antitumor responses following immunotherapy and chemotherapy for liver cancers, highlighting the therapeutic potential of gut microbiome modulation in enhancing treatment efficacy and reducing drug resistance. Fecal microbiota transplantation (FMT), a novel therapeutic modality to deliver a healthy donor's stool by endoscopy or capsule, has demonstrated potential in managing liver diseases and cancers by restoring and modulating the recipient's gut microbiome composition. However, existing data on the clinical application of FMT in liver cancers are still limited. This review summarizes the underlying roles and mechanisms of gut microbiome in liver cancer and discusses the therapeutic potential of FMT in liver cancer treatment and the management of its related complications (e.g., hepatic encephalopathy).

Keywords: Liver cancer, hepatocellular cancer, hepatic encephalopathy, gut microbiome, fecal microbiota transplantation

INTRODUCTION

Primary liver cancer is the sixth commonest cancer and the third leading cause of cancer mortality worldwide in 2020, with approximately 906,000 new cases and 830,000 deaths respectively[1]. Primary liver cancer includes 75%-85% of hepatocellular carcinoma (HCC), 10%-15% of intrahepatic cholangiocarcinoma(CCA), and some other rarer histological subtypes[1]. Hepatitis B, hepatitis C, heavy alcohol consumption, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, and smoking are major risk factors for HCC[2]. Although the molecular pathways leading to carcinogenesis in HCC are complex, recent evidence suggests that the gut microbiome plays a significant role in the progression of chronic liver disease and the subsequent development of HCC[3,4]. In addition, emerging evidence indicates that the gut microbiome may play a role in the host responding to antitumor treatment during immunotherapy and chemotherapy. This highlights the therapeutic potential of gut microbiome modulation in enhancing treatment efficacy and reducing drug resistance for liver cancer[3,4].

FMT is a novel treatment modality to modulate and restore the recipient's gut microbiome composition by transplanting the functional gut microbiota from healthy donors to patients[5]. FMT solution can be delivered into the recipient's gastrointestinal tract through various routes such as oral capsules, esophagogastroduodenoscopy, nasojejunal tube, rectal enema, and colonoscopy[6,7]. FMT has shown high efficacy in patients with recurrent and refractory *Clostridioides difficile* infection (CDI), which has now been recommended as a viable treatment option according to the United States, British, and European guidelines[8-11]. FMT has also demonstrated therapeutic potential in liver diseases, including alcoholic hepatitis[12], NAFLD[13], hepatitis B[14], and liver cirrhosis[15,16].

This review aims to summarize the current literature by exploring the mechanism of the gut microbiome in liver cancer and related complications and to discuss the recent advances in the role of FMT in antitumor therapies and responses.

DYSBIOSIS IN LIVER CANCERS

HCC is usually the sequelae of chronic liver disease (CLD). The majority of HCCs (80%-90%) are found in patients with advanced fibrosis or cirrhosis[17,18]. The development of HCC in patients with viral hepatitis is associated with persistent liver inflammation, but the precise mechanism in NAFLD cases remains unclear[19]. There is increasing evidence from both animal and human studies that suggests a connection between gut microbiota and the development of HCC. Changes in gut microbiome composition and microbiome-derived metabolites are thought to be responsible for promoting the progression of CLD and HCC occurrence[19,20].

It is widely acknowledged that dysbiosis occurs in HCC[21]. The α-diversity, which refers to the number of species present in each stool sample, has been found to be notably reduced in patients with HCC[22-24]. In addition, tissue samples from HCC also showed reduced α-diversity compared to patients with benign liver disease (e.g., hemangioma)[25]. At the phylum level, compared with the fecal microbiota of healthy individuals, the abundance of *Firmicutes* species was decreased. In contrast, that of *Proteobacteria* species was increased in both CLD and HCC patients[26]. In addition, the intrahepatic analysis indicated that HCC patients had a higher abundance of specific microbes in the *Stenotrophomonas* genus and the *Proteobacteria* phylum. This specific bacterium was very rare among the normal control group[25]. The dysbiosis in HCC also displayed a significant decrease in the relative abundance of short-chain fatty acids (SCFAs)-producing bacteria, such as *Ruminococcaceae*, *Butyricicoccus*, and *Lachnospiraceae*[27,28].

The etiologies of HCC can be divided into viral-related and non-viral-related, with different degrees of dysbiosis. Liu *et al.* compared the microbiota profile of HBV-related HCC (HBV-HCC) and non-HBV non-HCV related HCC (NBNC-HCC) and found that the HBV-HCC patients had a much higher level of species richness in fecal microbiota than NBNC-HCC patients[29]. An increase in the levels of proinflammatory bacteria (*Escherichia-Shigella*, *Enterococcus*) and a decrease in the levels of anti-inflammatory bacteria (*Faecalibacterium*, *Ruminococcus*, *Ruminoclostridium*) were observed in NBNC-HCC patients[29]. However, the bacterial composition of HBV-HCC patients was completely different from that of NBNC-HCC patients, with an increment in the levels of *Prevotella*, *Alloprevotella*, *Faecalibacterium*, *Ruminiclostridium* which were associated with anti-inflammatory potential[29]. The exact reason and clinical significance remain unknown.

Microbial dysbiosis has also been reported in different samples of patients with CCA[23,30-33]. An increased abundance of *Prevotella* species was identified in the oral, gut, and bile samples in patients with CCA[23]. In addition, the abundance of *Actinomyces* has been found to increase in the gut and bile[30] but decreased in oral samples in patients with CCA[32]. *Actinomyces* is associated with the disruption of mucosal membranes[34]. Zhang *et al.* found that the impaired gut barrier function could make gut-derived bacteria and lipopolysaccharide into hepatocytes, increasing polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) to create an immunosuppressive environment, which will promote liver carcinogenesis[35]. The increased *Actinomyces* in the gut might be associated with the impaired gut barrier function, leading to the increase of *Actinomyces* in bile and the association with CCA. Therefore, it is now believed that dysbiosis has a pivotal role in the pathogenesis of both HCC and CCA.

GUT MICROBIOME-RELATED PATHOGENESIS OF LIVER CANCERS AND COMPLICATIONS

The hepatic portal circulation is a process that transports nutrients from the intestines to the liver. During this process, the liver is exposed to metabolites and products derived from gut microbiota. This crosstalk between the gut and liver is termed as "gut-liver-axis"[3]. As alcoholic liver disease (ALD), NAFLD, liver cirrhosis, and associated complications are closely linked to increased bacterial translocation and dysbiosis[36,37], it is thought to be one of the key drivers that lead to hepatic microenvironment inflammation and progression toward cirrhosis and HCC[3,4].

Experiments in animal models have provided pre-clinical evidence that both microbiota-derived metabolites and their activated pathways may play a role in the development of HCC. Fox *et al.* investigated the role of a specific bacteria, *Helicobacter hepaticus*, in promoting tumorigenesis by chemicals or viruses in a transgenic mice model[38]. Their results showed that colonization with *H. hepaticus* in the gut could promote aflatoxin- and HCV transgene-induced HCC without hepatic bacterial translocation or induction of hepatitis[38]. In addition, the study from Dapito *et al.* using an HCC mice model induced by diethylnitrosamine (DEN) and hepatotoxin carbon tetrachloride (CCl4) further demonstrated that gut microbiota and Toll-like receptor 4 (TLR4) activation were essential in the hepatocarcinogenesis in chronically injured livers[39]. It was demonstrated by Zhang *et al.* that long-term DEN treatment led to a notable reduction in the level of *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* species and inflammation in the gut[20]. The presence of penicillin or dextran sulfate sodium (DSS)-induced intestinal dysbiosis or inflammation significantly increases the risk of tumor formation[20]. Supplementation with probiotics significantly alleviated gut dysbiosis, improved intestinal inflammation, and suppressed liver tumor growth and multiplicity[20]. Another experiment conducted by Yoshimoto *et al.* investigated obesity-related HCC by using a 7,12-dimethylbenz[a]anthracene (DMBA)-high-fat diet (HFD)-induced HCC mice model[40]. They found that obesity could lead to a rise in deoxycholic acid (DCA) levels. Elevated levels of DCA may trigger

the senescence-associated secretory phenotype (SASP) in hepatic stellate cells, in turn causing the release of inflammatory and tumor-promoting molecules in the liver, promoting the progression of HCC in mice[40]. Similarly, Zhang *et al.* used a high-fat/high-cholesterol (HFHC) diet-induced NAFLD-HCC model[41]. They were able to demonstrate that cholesterol-induced NAFLD-HCC formation was associated with gut microbiome dysbiosis. They also used germ-free mice receiving FMT products from donor mice fed with HFHC, which led to hepatic lipid accumulation, inflammation, and cell proliferation[41].

Epidemiological data showed that the incidence rates of HCC in males were two or three times higher than that in females[42], and it is well-acknowledged that sex hormones play a significant role in the gender disparity of HCC[43,44]. It is commonly considered that estrogens are protective, while androgens promote hepatocellular carcinogenesis[45]. Sex-specific enterotypes may influence the metabolism of hormones, subsequently affecting the development of HCC[46,47]. In addition, gut microbiota might be another source of sex hormones, as *Clostridium scindens* has been reported to convert glucocorticoids into androgens[48]. Mouse experiments showed that the bacteria involved in bile acid metabolism (e.g., *Clostridiales*, *Corynebacterium*, *Bacillus*, *Desulfovibrio*, *Rhodococcus*) were different between males and females, and the differences became more significant after induction of HCC model (streptozotocin-high fat diet)[49]. This study revealed that gut microbiota was involved in the sex-dependent effects on bile acid metabolism, which plays a pivotal role in the development of non-alcoholic steatohepatitis (NASH) and NASH-associated HCC[50,51]. On the other hand, Huang *et al.* studied the change of gut microbiota in male and female HCC mice models (i.e., liver-specific Tsc1 knockout mice)[52]. They found that the female mice had dysbiosis earlier than male mice in the process of developing HCC[52]. Specific bacteria associated with the risk of HCC in males (e.g., *Paraprevotella*, *Paraprevotellaceae*) and females (e.g., *Allobaculum*, *Erysipelotrichaceae*) were observed[52]. These studies based on animal models revealed the sex-dependent involvement of gut microbiota in the pathogenesis of HCC. More well-designed studies involving human or human-to-mice models to investigate the role of gut microbiota in the sexual dimorphism of HCC are needed.

The majority of HCC patients suffer from concurrent liver cirrhosis, which is not only the risk factor for liver cancers but also related complications (e.g., hepatic encephalopathy, HE)[53,54]. Concurrent HE can interfere with the antitumor therapy and thus negatively affect the prognosis of liver cancer. Multiple studies have demonstrated that the beneficial SCFAs-producing bacteria (i.e., *Lachnospiraceae* and *Ruminococcaceae*) were decreased, and the potentially pathogenic (i.e., *Enterobacteriaceae*) were increased in patients with HE[36,55-57]. This microbial profile has also been associated with cognitive impairment and the presence of systemic inflammation[57,58]. These emerging data support that the brain and the "gut-liver axis" are closely intertwined, and possibly these clinical manifestations are reflective of the "gut-liver-brain axis"[59]. The increasing understanding of the influence of gut microbiota on the development of liver cancers provides valuable insight into the use of microbiome modulation as a potential therapy for managing liver cancer and its associated complications.

THERAPEUTIC POTENTIAL OF MICROBIOME MODULATION IN ONCOLOGICAL TREATMENT FOR LIVER CANCER

Treatment options for liver cancer include surgical resection, chemotherapy, radiotherapy, radiofrequency ablation, hepatic artery chemoembolization, immunotherapy, and liver transplantation. In the case of advanced cancers, systemic therapy and multikinase inhibitors such as sorafenib and lenvatinib are the mainstay of treatment[60,61]. However, fewer than one-third of patients can benefit from these treatments as drug resistance and treatment-related adverse events are major roadblocks[62]. On the other hand, since 2017, the US Federal Drug Administration has approved immune checkpoint inhibitors (ICIs) as a second-line treatment for advanced HCC with sorafenib resistance. The combination of atezolizumab and

bevacizumab has proven superior to sorafenib[63]. An infusion regimen termed STRIDE (Single Tremelimumab Regular Interval Durvalumab) significantly improved overall survival versus sorafenib (36 months overall survival rate 30.7% *vs.* 20.2%)[64]. However, the overall success rate of ICI monotherapy or combination therapy has been reported to be only around 36%[65,66]. In addition, some patients develop drug resistance after treatment. Therefore, it would be beneficial if there is an adjunctive therapy to enhance the treatment efficacy and reduce the risk of drug resistance for chemotherapy and immunotherapy.

Chemotherapy and targeted agents

The gut microbiota modulates the host response to chemotherapeutic drugs in two major domains: drug efficacy and toxicity [Table 1][67]. Jin *et al.* found that gut microbiota can enhance the chemosensitivity of HCC to 5-fluorouracil *in vivo* by increasing curcumin bioavailability[68]. On the other hand, gut microbiota alteration may also affect drug toxicity and treatment-related adverse events (AEs). Inukai *et al.* compared the gut microbiota in patients who developed diarrhea and those who did not after receiving Lenvatinib treatment for HCC[69]. The group with diarrhea showed a higher relative abundance of *Parabacteroides* and *Prevotella*[69]. Similarly, Yamamoto *et al.* also found an increased relative abundance of *Butyricimonas*, a butyric acid-producing bacteria, in the asymptomatic group than in the group with diarrhea[70]. Butyrate has a vital role in suppressing inflammatory and allergic responses[70]. Based on the above preliminary findings, chemotherapy combined with adjunctive microbiota modulation may be a promising therapeutic approach.

Immunotherapy

Immunotherapy is a promising oncological treatment by modulating the interaction between the host's immune system and cancer cells. Emerging evidence demonstrates the close crosstalk between the gut microbiota and patients' response to ICIs [Table 1]. For example, the role of gut microbiota in modulating treatment response among patients on ICI targeting programmed cell death protein 1 (anti-PD-1) has garnered increasing attention in recent years[71,72]. Zheng *et al.* collected stool samples in HCC patients who received immunotherapy and revealed that responders had higher taxa richness and more gene counts than non-responders[73]. Responders were enriched with some beneficial species, such as *Akkermansia muciniphila* and *Ruminococcaceae spp*[73]. Similarly, Chung *et al.* proposed that a skewed *Firmicutes/Bacteroidetes* ratio and a low *Prevotella/Bacteroides* ratio could be predictive markers of non-responders among HCC patients receiving ICIs[74]. In contrast, the presence of *Akkermansia* species predicted a good response[74]. Wu *et al.* also found that responders had a higher level of α-diversity at baseline than non-responders[75]. They also profiled the serum metabolites and found that responders were enriched with *Ruminococcus* which was positively correlated with serum galactaric acid[75]. Furthermore, Routy *et al.* performed FMT on germ-free or antibiotic-treated mice using fecal samples from epithelial cancer patients who responded to ICIs and found that it could show the antitumor effects of PD-1 blockade, while FMT from non-responders could not restitute the same effect[76]. In a subgroup of patients with primary PD-1-refractory melanoma, FMT using a donor from the anti-PD1 responder could overcome drug resistance and re-capture anti-PD1 treatment response among 40% (6/15) of them by re-programming the gut microbiome and tumor microenvironment[77]. Metagenomics analysis revealed correlations between the relative abundance of *A. muciniphila* and clinical responses to ICIs[76]. Oral administration of *A. muciniphila* after FMT in non-responders has been shown to restore the efficacy of PD-1 blockade[76]. In addition, microbiota-derived metabolites also showed an association with clinical outcomes in patients with HCC receiving ICIs[78]. Responders had a significantly higher level of secondary bile acids[78]. If these findings can be replicated, modulating gut microbiota before immunotherapy by FMT from selected donors enriched in certain favorable species may potentially increase the clinical response rate of immunotherapy for liver cancer. In addition, subgroup analyses of survival outcomes according to clinical trials which evaluated the efficacy of ICIs revealed a discrepancy between viral HCC and non-viral HCC[79-82], implying a potential influence of tumor etiologies on treatment response[83]. Considering the variation in dysbiosis[29], more

Table 1. Gut microbiome alteration in antitumor therapies for liver cancer

Reference	Models	Therapies	Diseases	Implicated microbiota	Sequencing method
Inukai *et al.*[69]	Human	Chemotherapy	HCC (diarrhea and non-diarrhea)	↑ *Parabacteroides* and *Prevotella* in the diarrhea group	16S rRNA gene sequencing
Yamamoto *et al.*[70]	Human	Chemotherapy	HCC (diarrhea and non-diarrhea)	↑ *Butyricimonas*, ↓ *Citrobacter*, *Peptostreptococcus*, and *Staphylococcaceae* in non-diarrhea group	16S rRNA gene sequencing
Jin *et al.*[68]	Mice	Chemotherapy (curcumin combined with 5-fluorouracil)	HCC	↑ Richness (Chao 1 index); curcumin treatment significantly ↑ *Bifidobacterium* and *Lactobacillus* in gut	16S rDNA sequencing
Wu *et al.*[75]	Human	Immunotherapy (anti-PD-1)	HCC (R and NR)	↑ α-diversity (Shannon and inverse Simpson indexes) in R; ↑ *Faecalibacterium*, *Blautia*, *Lachnospiracea incertae Sedis*, *Megamonas*, *Ruminococcus*, *Coprococcus*, *Dorea* and *Haemophilus* in R; ↑ *Atopobium*, *Leptotrichia*, *Campylobacter*, *Allisonella*, *Methanobrevibacter*, *Parabacteroides*, *Bifidobacterium,* and *Lactobacillus* in NR	16S rRNA gene sequencing
Lee *et al.*[78]	Human	Immunotherapy (ICIs)	HCC (R and NR)	↑ *Prevotella 9* in NR, ↑ *Lachnoclostridium*, *Lachnospiraceae*, and *Veillonella* in R; ↑*Lachnoclostridium* and ↓ *Prevotella 9* were associated with better overall survival	16S rRNA gene sequencing
Chung *et al.*[74]	Human	Immunotherapy (nivolumab)	HCC (R and NR)	NR: a skewed Firmicutes/Bacteroidetes ratio and a ↓ *Prevotella/Bacteroides* ratio; presence of *Akkermansia* species was associated with good response	16S rRNA gene sequencing
Shen *et al.*[108]	Human	Immunotherapy (ICIs)	HCC (R and NR)	↑*Bifidobacterium*, *Coprococcus*, and *Acidaminococcus*- in patients with disease control	16S rRNA gene sequencing
Zheng *et al.*[73]	Human	Immunotherapy (anti-PD-1 antibodies)	HCC	↑ Four *Lactobacillus* species (*L. oris, L. mucosae, L. gasseri,* and *L. vaginalis*), *Bifidobacterium dentium* and *Streptococcus thermophilus* in R	Metagenomic sequencing
Mao *et al.*[109]	Human	Immunotherapy (anti-PD-1)	HCC or advanced biliary tract cancers	↑ *Ruminococcus calidus* and *Erysipelotichaceae bacterium-GAM147* in response. ↑ *Veillonellaceae* in NR	Metagenomic sequencing
Li *et al.*[84]	Human and mice	Radiotherapy	HCC (R and NR)	↓ Diversity in NR; the distribution of the R group samples was closer to healthy control group samples; At the genus level, ↑ *Faecalibacterium* was observed in the healthy control and R group; ↑ order *Clostridiales*, family *Ruminococcaceae*, and genus *Faecalibacterium* in the R group; ↑ order *Lactobacillales* in the NR group	16S rRNA sequencing
Bian *et al.*[86]	Rabbit	Transarterial chemoembolization	HCC	↑*Ruminococcus* and *Roseburia*; ↓ *Bacteroides*; *Parabacteroides* and *Escherichia* after surgery	qPCR

HCC: hepatocellular cancer; PHLF: post-hepatectomy liver failure; R: responder; NR: non-responder; ICIs: immune checkpoint inhibitors; qPCR: quantitative polymerase chain reaction.

studies are needed to support the gut microbiome modulation before immunotherapy based on the etiologies of HCC.

Locoregional therapies

The role of gut microbiota in other locoregional treatments for liver cancer has also attracted considerable interest recently [Table 1]. Li *et al*. analyzed the fecal microbiota from HCC patients who received radiotherapy and found that the distribution of microbiota from responders was closer to that found in healthy controls[84]. Responders were enriched with specific bacteria at the genus level, such as *Faecalibacteriu*, while non-responders had a high abundance of members of the genus *Streptococcus*[84]. Further animal experiments demonstrated that gut dysbiosis induced by antibiotics significantly impaired

radiotherapy-induced T-cell infiltration and that FMT could restore the antitumor effects of radiotherapy[84].

Another treatment option for intermediate and advanced liver cancer is transarterial chemoembolization (TACE), using localized high concentrations of chemotherapy agents and embolizing feeding vessels by oil iodide emulsion[85]. Bian *et al.* conducted TACE in rabbit HCC models and found that the procedure partially reversed the tumor-induced dysbiosis, improving the intestinal barrier and liver functions and decreasing the lipopolysaccharide (LPS) level in the blood[86]. Some key operational taxonomic units (OTUs) were closely associated with clinical factors, which provides a theoretical basis for the therapy combination of TACE with microbiota-targeted intervention in the future[86].

FMT IN LIVER CANCERS AND RELATED COMPLICATIONS

Endoscopic procedures of FMT

FMT can be delivered into patients' gastrointestinal tract via several routes, such as esophagogastroduodenoscopy (EGD), colonoscopy, feeding tubes, or rectal enemas[87]. Fresh or frozen fecal suspensions can be delivered into the duodenum or colon through the working channel of the flexible endoscope. Caution needs to be exercised when FMT is given by EGD in patients with a history of stomach or duodenal surgery to avoid adverse events such as aspiration[88]. FMT through colonoscopy allows for the concomitant diagnosis of colonic diseases and may be a preferable route in some situations[89,90].

Current evidence of FMT in liver cancers and related complications

To date, published data on FMT in liver cancer treatment is unavailable. Yet, it is worth noting that there is a recently registered clinical trial to investigate the effect of FMT in overcoming drug resistance to atezolizumab/bevacizumab among patients with liver cancers, which has been registered in ClinicalTrials.gov (NCT05690048) [Table 2]. Apart from atezolizumab and bevacizumab, the intervention group will receive three days of oral vancomycin followed by two rounds of FMT capsules, compared with placebo capsules in the control group. We eagerly await the results of this trial.

On the other hand, more published data are available to demonstrate the efficacy and safety of FMT in liver cancer-related complications [Table 2]. FMT is now a potential therapeutic option for HE. Bajaj *et al.* conducted a randomized controlled trial (RCT) investigating the safety and efficacy of FMT from donors with high levels of *Lachnospiraceae* and *Ruminococcaceae*[15]. The results showed that patients receiving a single FMT enema developed less severe adverse events (SAE) (20% *vs.* 80%) and less recurrent HE (0% *vs.* 50%) compared with the control group (who were given the standard of care)[15]. Two SAEs (i.e., hospitalization) in the FMT group were considered unrelated to the FMT by the independent Data Safety Monitoring Board[15]. Furthermore, FMT group showed improved cognition, but the control group did not[15]. Microbiome analysis indicated that, after FMT, a significant increase was found in the relative abundance of *Lachnospiraceaeae* and *Ruminococcaceaen*[15]. Based on these findings, Bajaj *et al.* conducted another RCT treating patients with HE by FMT capsules from a single donor who had enriched *Lachnospiraceaeae* and *Ruminococcaceae*, demonstrating that oral FMT capsules were safe and well-tolerated even in patients with cirrhosis and recurrent HE[16]. This trial also confirmed the efficacy of FMT in improving duodenal mucosal diversity, dysbiosis, and resolution of HE. Similarly, a recent study investigated the efficacy and safety of FMT capsules from different donors in HE[91]. They found that the levels of *Bifidobacterium* and other known beneficial bacteria at baseline and throughout the study were higher in responders[91]. Moreover, the fecal SCFAs levels from donors were the lowest among recipients with worse cognitive outcomes[91]. These data suggested that the efficacy of FMT can be affected by recipient and donor effects. More data exploring the crosstalk between the microbiome of donor and recipient are needed before more definitive conclusions can be drawn.

Table 2. Application of FMT in liver cancer and related complications

Reference	Model /study type	Disease	FMT details	Clinical outcome	Implicated microbiota
Clinicaltrial.gov: NCT05690048	Human/phase II, single blind, RCT,	Liver cancer (unresectable HCC, BCLC stage C)	FMT via capsule (50 g of fecal matter) on day 0 and day 21	The primary outcomes are differential tumoral CD8 T-cell infiltration and adverse event documentation of FMT	NA
Clinicaltrial.gov: NCT05170971	Human/open label	Liver failure	Four times of FMT every 5 days, follow-up for 2 months	The primary outcomes are the change in liver function, coagulation function, MELD score, clinical manifestations, safety and adverse events and change of proinflammatory cytokine	The change in gut microbiota will be checked
Bloom *et al.*[91]	Human/open label (10 subjects)	HE	FMT capsules 5 times over 3 weeks, follow-up for 6 months	The PHES improved after three doses and five doses of FMT, and four weeks after the fifth dose of FMT. One SAE was extended spectrum beta lactamase *Escherichia coli* bacteremia	Responders had higher levels of *Bifidobacterium* and other beneficial species at baseline and throughout the study
Bajaj *et al.*[16]	Human/phase 1, RCT (20 subjects)	HE	15 FMT capsules (universal donor with high *Lachnospiraceae* and *Ruminococcaceae* relative abundance), follow up for 5 months	All subjects tolerated the procedures (EGD, sigmoidoscopy and capsule); ↓ SAEs and number of patients with SAEs in the FMT group; ↑ improvement in brain function in the FMT group compared to baseline	In the duodenum, ↑ *Ruminococcaceae* and *Bifidobacteriaceae* and ↓ *Veillonellaceae* and *Streptococcaceae* post-FMT compared to pre-FMT
Bajaj *et al.*[15]	Human/RCT (20 subjects)	HE	Three frozen FMT units (90 mL total) were administered by enema and retained for 30 min, follow-up for five months	↓SAE in FMT group compared to SOC group (8 *vs.* 2); Five SOC group and no FMT participants developed further HE. Cognition improved in FMT, but not SOC group	↑ *Lactobacillaceae*, *Bifidobacteriaceae* post-FMT; patients in the FMT arm showed ↑ *Lachnospiraceaeae* and *Ruminococcaceae* after FMT
Sharma *et al.*[110]	Human/open label (13 subjects)	Alcohol-associated acute-on-chronic liver failure	FMT through nasojejunal tube from selected family members, follow up 3 months	Survival at 28 and 90 days was significantly better in the FMT arm (100% *vs.* 60%; 53.84% *vs.* 25%). More HE and ascites resolved in the FMT group compared to the SOC group (100% *vs.* 57.14%; 100% *vs.* 40%). Adverse events were similar in both groups	NA
Huang *et al.*[111]	Rats	portal hypertension and portosystemic collaterals	FMT by oral gavage	FMT ↓ portal pressure in cirrhotic rats	↓*Lachnospiraceae* in cirrhotic rats. FMT ↑ *Bifidobacterium*

FMT: fecal microbiota transplantation; BCLC: Barcelona Clinic Liver Cancer; HE: hepatic encephalopathy; MELD: model for end-stage liver disease; PHES: psychometric hepatic encephalopathy score; SAEs: severe adverse events; RCT: randomized control trial; SOC: standard of care; EGD: Esophagogastroduodenoscopy.

LIMITATIONS AND FUTURE PERSPECTIVE

Although the safety of FMT has been demonstrated in patients with CDI[90,92,93], inflammatory bowel diseases (IBD)[94-98], other types of cancers[77,99,100], graft versus host disease (GVHD)[101,102], and critically ill patients hospitalized in the intensive care unit (ICU)[103], safety issues relating to FMT in patients with liver cirrhosis and liver cancer should be taken into consideration. One SAE of extended-spectrum beta-lactamase (ESBL) *Escherichia coli* bacteremia has been reported previously[91]. Portal hypertension, which results in increased intestinal permeability and impaired barrier function, may induce additional risks to bacterial translocation, leading to an excessive risk of bacteremia. Recently, a new methodology based on an automatic purification system of FMT, known as washed microbiota transplantation (WMT), showed lower AE rates than traditional manual methods[97,104], WMT was regarded as a safer and cleaner type of

FMT[105,106], which could be an alternative option in high-risk patients with liver cancers and related complications. In addition to the safety issues, cost-effectiveness is another critical area worth further study. A multidisciplinary team, including endoscopists, hepatologists, oncologists, microbiologists, pharmacists, and other related healthcare professionals, can work together to develop an appropriate and pragmatic strategy to help these patients. The primary diagnosis, cancer stage, complications, microbiota profiles, and oncological therapies should be considered on an individualized basis to determine the optimal delivery route, dose, and frequency of FMT. Selective microbiota transplantation (SMT) by matching selected donors could be considered to reduce the potential infective risks further. Under the premise of ensuring safety, FMT and other microbiome modulation modalities can be considered adjunctive therapy for liver cancer and related complications[107].

CONCLUSION

In conclusion, the role of gut microbiota modulation in patients with liver cancer and related complications is biologically plausible and supported by a growing body of literature. More studies on efficacy and safety are needed if FMT is to be incorporated into mainstream clinical applications.

DECLARATIONS

Authors' contributions

Did literature review, drafted and revised the manuscript: Dai M
Revised the manuscript: Lau LHS, Lui RN

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Lau LHS has received research grant support from GenieBiome Ltd. Lau LHS has served as a lecture speaker for Olympus, Boston Scientific, Pfizer, and GenieBiome Ltd. Lui NR has served as an advisory board member for Gilead Sciences and as a speaker for GenieBiome, Gilead Sciences, and Pierre Fabre, and owns equity in Pfizer.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71:209-49. DOI
2. McGlynn KA, Petrick JL, London WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. *Clin Liver Dis* 2015;19:223-38. DOI PubMed PMC
3. Tripathi A, Debelius J, Brenner DA, et al. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018;15:397-411. DOI

4. Yu LX, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. *Nat Rev Gastroenterol Hepatol* 2017;14:527-39. DOI PubMed PMC
5. Gupta A, Khanna S. Fecal microbiota transplantation. *JAMA* 2017;318:102. DOI PubMed
6. Peng Z, Xiang J, He Z, et al. Colonic transendoscopic enteral tubing: a novel way of transplanting fecal microbiota. *Endosc Int Open* 2016;4:E610-3. DOI PubMed PMC
7. Wang W, Lu G, Wu X, Wen Q, Zhang F. Colonic transendoscopic enteral tubing is a new pathway to microbial therapy, colonic drainage, and host-microbiota interaction research. *J Clin Med* 2023;12:780. DOI PubMed PMC
8. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013;368:407-15. DOI PubMed
9. Johnson S, Lavergne V, Skinner AM, et al. Clinical practice guideline by the infectious diseases society of America (IDSA) and society for healthcare epidemiology of America (SHEA): 2021 focused update guidelines on management of clostridioides difficile infection in adults. *Clin Infect Dis* 2021;73:755-7. DOI
10. Mullish BH, Quraishi MN, Segal JP, et al. The use of faecal microbiota transplant as treatment for recurrent or refractory Clostridium difficile infection and other potential indications: joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut* 2018;67:1920-41. DOI PubMed
11. Prehn J, Reigadas E, Vogelzang EH, et al; Guideline Committee of the European Study Group on Clostridioides difficile. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for Clostridioides difficile infection in adults. *Clin Microbiol Infect* 2021;27 Suppl 2:S1-S21. DOI
12. Xue L, Deng Z, Luo W, He X, Chen Y. Effect of fecal microbiota transplantation on non-alcoholic fatty liver disease: a randomized clinical trial. *Front Cell Infect Microbiol* 2022;12:759306. DOI PubMed PMC
13. Craven L, Rahman A, Nair Parvathy S, et al. Allogenic fecal microbiota transplantation in patients with nonalcoholic fatty liver disease improves abnormal small intestinal permeability: a randomized control trial. *Am J Gastroenterol* 2020;115:1055-65. DOI
14. Chauhan A, Kumar R, Sharma S, et al. Fecal microbiota transplantation in hepatitis B e antigen-positive chronic hepatitis b patients: a pilot study. *Dig Dis Sci* 2021;66:873-80. DOI
15. Bajaj JS, Kassam Z, Fagan A, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. *Hepatology* 2017;66:1354-5. DOI
16. Bajaj JS, Salzman NH, Acharya C, et al. Fecal microbial transplant capsules are safe in hepatic encephalopathy: a phase 1, randomized, placebo-controlled trial. *Hepatology* 2019;70:1690-703. DOI PubMed PMC
17. Cabrera R, Nelson DR. Review article: the management of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2010;31:461-76. DOI PubMed
18. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011;365:1118-27. DOI PubMed
19. Schwabe RF, Greten TF. Gut microbiome in HCC-mechanisms, diagnosis and therapy. *J Hepatol* 2020;72:230-8. DOI PubMed
20. Zhang HL, Yu LX, Yang W, et al. Profound impact of gut homeostasis on chemically-induced pro-tumorigenic inflammation and hepatocarcinogenesis in rats. *J Hepatol* 2012;57:803-12. DOI
21. Das BK. Altered gut microbiota in hepatocellular carcinoma: Insights into the pathogenic mechanism and preclinical to clinical findings. *APMIS* 2022;130:719-40. DOI PubMed
22. Ni J, Huang R, Zhou H, et al. Analysis of the relationship between the degree of dysbiosis in gut microbiota and prognosis at different stages of primary hepatocellular carcinoma. *Front Microbiol* 2019;10:1458. DOI PubMed PMC
23. Deng T, Li J, He B, et al. Gut microbiome alteration as a diagnostic tool and associated with inflammatory response marker in primary liver cancer. *Hepatol Int* 2022;16:99-111. DOI
24. Lai MW, Chu YD, Hsu CW, Chen YC, Liang KH, Yeh CT. Multi-omics analyses identify signatures in patients with liver cirrhosis and hepatocellular carcinoma. *Cancers* 2022;15:210. DOI PubMed PMC
25. Liu B, Zhou Z, Jin Y, 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. DOI PubMed PMC
26. Chen T, Ding R, Chen X, et al. Firmicutes and blautia in gut microbiota lessened in chronic liver diseases and hepatocellular carcinoma patients: a pilot study. *Bioengineered* 2021;12:8233-46. DOI PubMed PMC
27. Lapidot Y, Amir A, Nosenko R, et al. Alterations in the gut microbiome in the progression of cirrhosis to hepatocellular carcinoma. *mSystems* 2020;5:e00153-20. DOI PubMed PMC
28. Ren Z, Li A, Jiang J, et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut* 2019;68:1014-23. DOI PubMed PMC
29. Liu Q, Li F, Zhuang Y, et al. Alteration in gut microbiota associated with hepatitis B and non-hepatitis virus related hepatocellular carcinoma. *Gut Pathog* 2019;11:1. DOI PubMed PMC
30. Jia X, Lu S, Zeng Z, et al. Characterization of gut microbiota, bile acid metabolism, and cytokines in intrahepatic cholangiocarcinoma. *Hepatology* 2020;71:893-906. DOI
31. Saab M, Mestivier D, Sohrabi M, et al. Characterization of biliary microbiota dysbiosis in extrahepatic cholangiocarcinoma. *PLoS One* 2021;16:e0247798. DOI PubMed PMC
32. Rao BC, Zhang GZ, Zou YW, et al. Alterations in the human oral microbiome in cholangiocarcinoma. *Mil Med Res* 2022;9:62. DOI PubMed PMC
33. Chen B, Fu SW, Lu L, Zhao H. A preliminary study of biliary microbiota in patients with bile duct stones or distal

cholangiocarcinoma. *Biomed Res Int* 2019;2019:1092563. DOI PubMed PMC
34. Pujic P, Beaman BL, Ravalison M, Boiron P, Rodríguez-nava V. Nocardia and actinomyces. Molecular medical microbiology. Elsevier; 2015. pp. 731-52. DOI
35. Zhang Q, Ma C, Duan Y, et al. Gut microbiome directs hepatocytes to recruit MDSCs and promote cholangiocarcinoma. *Cancer Discov* 2021;11:1248-67. DOI PubMed PMC
36. Bajaj JS, Heuman DM, Hylemon PB, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014;60:940-7. DOI PubMed PMC
37. Shen F, Zheng RD, Sun XQ, Ding WJ, Wang XY, Fan JG. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int* 2017;16:375-81. DOI PubMed
38. Fox JG, Feng Y, Theve EJ, et al. Gut microbes define liver cancer risk in mice exposed to chemical and viral transgenic hepatocarcinogens. *Gut* 2010;59:88-97. DOI PubMed PMC
39. Dapito DH, Mencin A, Gwak GY, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012;21:504-16. DOI PubMed PMC
40. Yoshimoto S, Loo TM, Atarashi K, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97-101. DOI
41. Zhang X, Coker OO, Chu ES, et al. Dietary cholesterol drives fatty liver-associated liver cancer by modulating gut microbiota and metabolites. *Gut* 2021;70:761-74. DOI PubMed PMC
42. Petrick JL, Florio AA, Znaor A, et al. International trends in hepatocellular carcinoma incidence, 1978-2012. *Int J Cancer* 2020;147:317-30. DOI PubMed PMC
43. Yeh SH, Chen PJ. Gender disparity of hepatocellular carcinoma: the roles of sex hormones. *Oncology* 2010;78 Suppl 1:172-9. DOI PubMed
44. Nevola R, Tortorella G, Rosato V, et al. Gender differences in the pathogenesis and risk factors of hepatocellular carcinoma. *Biology* 2023;12:984. DOI PubMed PMC
45. Kalra M, Mayes J, Assefa S, Kaul AK, Kaul R. Role of sex steroid receptors in pathobiology of hepatocellular carcinoma. *World J Gastroenterol* 2008;14:5945-61. DOI PubMed PMC
46. Markle JG, Frank DN, Mortin-Toth S, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 2013;339:1084-8. DOI
47. Yurkovetskiy L, Burrows M, Khan AA, et al. Gender bias in autoimmunity is influenced by microbiota. *Immunity* 2013;39:400-12. DOI PubMed PMC
48. Ridlon JM, Ikegawa S, Alves JM, et al. Clostridium scindens: a human gut microbe with a high potential to convert glucocorticoids into androgens. *J Lipid Res* 2013;54:2437-49. DOI PubMed PMC
49. Xie G, Wang X, Zhao A, et al. Sex-dependent effects on gut microbiota regulate hepatic carcinogenic outcomes. *Sci Rep* 2017;7:45232. DOI PubMed PMC
50. Zhou J, Tripathi M, Sinha RA, Singh BK, Yen PM. Gut microbiota and their metabolites in the progression of non-alcoholic fatty liver disease. *Hepatoma Res* 2021;7:11. DOI PubMed PMC
51. Sousa P, Machado MV. MAFLD under the lens: the role of gut microbiota. *Metab Target Organ Damage* 2022;2:14. DOI
52. Huang R, Li T, Ni J, et al. Different sex-based responses of gut microbiota during the development of hepatocellular carcinoma in liver-specific Tsc1-knockout mice. *Front Microbiol* 2018;9:1008. DOI PubMed PMC
53. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004;127:S35-50. DOI PubMed
54. Meriggi F, Graffeo M. Clinical characterisation and management of the main treatment-induced toxicities in patients with hepatocellular carcinoma and cirrhosis. *Cancers* 2021;13:584. DOI PubMed PMC
55. Chen Y, Yang F, Lu H, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011;54:562-72. DOI
56. Bajaj JS, Fagan A, White MB, et al. Specific gut and salivary microbiota patterns are linked with different cognitive testing strategies in minimal hepatic encephalopathy. *Am J Gastroenterol* 2019;114:1080-90. DOI PubMed PMC
57. Bajaj JS, Shamsaddini A, Fagan A, et al. Distinct gut microbial compositional and functional changes associated with impaired inhibitory control in patients with cirrhosis. *Gut Microbes* 2021;13:1953247. DOI PubMed PMC
58. Bajaj JS, Ridlon JM, Hylemon PB, et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G168-75. DOI PubMed PMC
59. Ho AHY, Wong S, Lui R. Topic: nutrition and the gut-liver-brain axis. *Curr Hepatology Rep* 2022;21:99-110. DOI
60. Jackson R, Psarelli EE, Berhane S, Khan H, Johnson P. Impact of viral status on survival in patients receiving sorafenib for advanced hepatocellular cancer: a meta-analysis of randomized phase III trials. *J Clin Oncol* 2017;35:622-8. DOI PubMed
61. Kudo M, Finn RS, Qin S, 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. DOI
62. El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology* 2008;134:1752-63. DOI PubMed
63. Finn RS, Qin S, Ikeda M, et al; IMbrave150 Investigators. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* 2020;382:1894-905. DOI

64. Abou-alfa GK, Lau G, Kudo M, et al. Tremelimumab plus durvalumab in unresectable hepatocellular carcinoma. *NEJM Evidence* 2022;1:EVIDoa2100070. DOI
65. Yau T, Kang YK, Kim TY, et al. Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: the checkmate 040 randomized clinical trial. *JAMA Oncol* 2020;6:e204564. DOI PubMed PMC
66. Finn RS, Ikeda M, Zhu AX, et al. Phase Ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. *J Clin Oncol* 2020;38:2960-70. DOI PubMed PMC
67. Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nat Rev Gastroenterol Hepatol* 2017;14:356-65. DOI PubMed
68. Jin M, Kong L, Han Y, Zhang S. Gut microbiota enhances the chemosensitivity of hepatocellular carcinoma to 5-fluorouracil *in vivo* by increasing curcumin bioavailability. *Phytother Res* 2021;35:5823-37. DOI PubMed
69. Inukai Y, Yamamoto K, Honda T, et al. Differences in the intestinal microbiome associated with diarrhea during lenvatinib treatment for hepatocellular carcinoma. *Dig Dis* 2023;41:138-47. DOI PubMed PMC
70. Yamamoto K, Kuzuya T, Honda T, et al. Relationship between adverse events and microbiomes in advanced hepatocellular carcinoma patients treated with sorafenib. *Anticancer Res* 2020;40:665-76. DOI
71. El-Khoueiry AB, Sangro B, Yau T, 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. DOI PubMed PMC
72. Zhu AX, Finn RS, Edeline J, et al; KEYNOTE-224 investigators. 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. DOI PubMed
73. Zheng Y, Wang T, Tu X, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. *J Immunother Cancer* 2019;7:193. DOI PubMed PMC
74. Chung MW, Kim MJ, Won EJ, et al. Gut microbiome composition can predict the response to nivolumab in advanced hepatocellular carcinoma patients. *World J Gastroenterol* 2021;27:7340-9. DOI PubMed PMC
75. Wu H, Zheng X, Pan T, et al. Dynamic microbiome and metabolome analyses reveal the interaction between gut microbiota and anti-PD-1 based immunotherapy in hepatocellular carcinoma. *Int J Cancer* 2022;151:1321-34. DOI PubMed
76. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018;359:91-7. DOI PubMed
77. Davar D, Dzutsev AK, McCulloch JA, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* 2021;371:595-602. DOI PubMed PMC
78. Lee PC, Wu CJ, Hung YW, et al. Gut microbiota and metabolites associate with outcomes of immune checkpoint inhibitor-treated unresectable hepatocellular carcinoma. *J Immunother Cancer* 2022;10:e004779. DOI PubMed PMC
79. Finn RS, Ryoo BY, Merle P, et al; KEYNOTE-240 investigators. 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. DOI
80. Kelley RK, Sangro B, Harris W, et al. Safety, efficacy, and pharmacodynamics of tremelimumab plus durvalumab for patients with unresectable hepatocellular carcinoma: randomized expansion of a phase I/II study. *J Clin Oncol* 2021;39:2991-3001. DOI PubMed PMC
81. Yau T, Park J, Finn R, et al. CheckMate 459: a randomized, multi-center phase III study of nivolumab (NIVO) *vs.* sorafenib (SOR) as first-line (1L) treatment in patients (pts) with advanced hepatocellular carcinoma (aHCC). *Annals of Oncology* 2019;30:v874-5. DOI
82. Abou-alfa GK, Chan SL, Kudo M, et al. Phase 3 randomized, open-label, multicenter study of tremelimumab (T) and durvalumab (D) as first-line therapy in patients (pts) with unresectable hepatocellular carcinoma (uHCC): HIMALAYA. *J Clin Oncol* 2022;40:379. DOI
83. Costante F, Airola C, Santopaolo F, Gasbarrini A, Pompili M, Ponziani FR. Immunotherapy for nonalcoholic fatty liver disease-related hepatocellular carcinoma: lights and shadows. *World J Gastrointest Oncol* 2022;14:1622-36. DOI PubMed PMC
84. Li Z, Zhang Y, Hong W, et al. Gut microbiota modulate radiotherapy-associated antitumor immune responses against hepatocellular carcinoma via STING signaling. *Gut Microbes* 2022;14:2119055. DOI PubMed PMC
85. McIlwain DR, Lang PA, Maretzky T, et al. iRhom2 regulation of TACE controls TNF-mediated protection against Listeria and responses to LPS. *Science* 2012;335:229-32. DOI PubMed PMC
86. Bian CF, Wang Y, Yu A, et al. Gut microbiota changes and biological mechanism in hepatocellular carcinoma after transarterial chemoembolization treatment. *Front Oncol* 2022;12:1002589. DOI PubMed PMC
87. Gulati M, Singh SK, Corrie L, Kaur IP, Chandwani L. Delivery routes for faecal microbiota transplants: available, anticipated and aspired. *Pharmacol Res* 2020;159:104954. DOI
88. Baxter M, Ahmad T, Colville A, Sheridan R. Fatal aspiration pneumonia as a complication of fecal microbiota transplant. *Clin Infect Dis* 2015;61:136-7. DOI PubMed
89. Kelly CR, Khoruts A, Staley C, et al. Effect of fecal microbiota transplantation on recurrence in multiply recurrent clostridium difficile infection: a randomized trial. *Ann Intern Med* 2016;165:609-16. DOI PubMed PMC
90. Kao D, Roach B, Silva M, et al. Effect of oral capsule- *vs* colonoscopy-delivered fecal microbiota transplantation on recurrent clostridium difficile infection: a randomized clinical trial. *JAMA* 2017;318:1985-93. DOI PubMed PMC
91. Bloom PP, Donlan J, Torres Soto M, Daidone M, Hohmann E, Chung RT. Fecal microbiota transplant improves cognition in hepatic

encephalopathy and its effect varies by donor and recipient. *Hepatol Commun* 2022;6:2079-89. DOI PubMed PMC

92. Hvas CL, Dahl Jørgensen SM, Jørgensen SP, et al. Fecal microbiota transplantation is superior to fidaxomicin for treatment of recurrent clostridium difficile infection. *Gastroenterology* 2019;156:1324-1332.e3. DOI
93. Juul FE, Garborg K, Bretthauer M, et al. Fecal microbiota transplantation for primary clostridium difficile infection. *N Engl J Med* 2018;378:2535-6. DOI PubMed
94. Haifer C, Paramsothy S, Kaakoush NO, et al. Lyophilised oral faecal microbiota transplantation for ulcerative colitis (LOTUS): a randomised, double-blind, placebo-controlled trial. *Lancet Gastroenterol Hepatol* 2022;7:141-51. DOI
95. Sokol H, Landman C, Seksik P, et al; Saint-Antoine IBD Network. Fecal microbiota transplantation to maintain remission in Crohn's disease: a pilot randomized controlled study. *Microbiome* 2020;8:12. DOI PubMed PMC
96. Costello SP, Hughes PA, Waters O, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. *JAMA* 2019;321:156-64. DOI PubMed PMC
97. Wang H, Cui B, Li Q, et al. The safety of fecal microbiota transplantation for Crohn's disease: findings from a long-term study. *Adv Ther* 2018;35:1935-44. DOI PubMed PMC
98. Paramsothy S, Kamm MA, Kaakoush NO, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet* 2017;389:1218-28. DOI
99. Baruch EN, Youngster I, Ben-Betzalel G, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* 2021;371:602-9. DOI
100. Ianiro G, Rossi E, Thomas AM, et al. Faecal microbiota transplantation for the treatment of diarrhoea induced by tyrosine-kinase inhibitors in patients with metastatic renal cell carcinoma. *Nat Commun* 2020;11:4333. DOI PubMed PMC
101. Kakihana K, Fujioka Y, Suda W, et al. Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. *Blood* 2016;128:2083-8. DOI PubMed PMC
102. Zhao Y, Li X, Zhou Y, et al. Safety and efficacy of fecal microbiota transplantation for grade IV steroid refractory GI-GvHD patients: interim results from FMT2017002 trial. *Front Immunol* 2021;12:678476. DOI PubMed PMC
103. Dai M, Liu Y, Chen W, et al. Rescue fecal microbiota transplantation for antibiotic-associated diarrhea in critically ill patients. *Crit Care* 2019;23:324. DOI PubMed PMC
104. Ding X, Li Q, Li P, et al. Long-term safety and efficacy of fecal microbiota transplant in active ulcerative colitis. *Drug Saf* 2019;42:869-80. DOI
105. Zhang T, Lu G, Zhao Z, et al. Washed microbiota transplantation *vs*. manual fecal microbiota transplantation: clinical findings, animal studies and *in vitro* screening. *Protein Cell* 2020;11:251-66. DOI PubMed PMC
106. Lu G, Wang W, Li P, Wen Q, Cui B, Zhang F. Washed preparation of faecal microbiota changes the transplantation related safety, quantitative method and delivery. *Microb Biotechnol* 2022;15:2439-49. DOI PubMed PMC
107. Xiang Z, Wu J, Li J, Zheng S, Wei X, Xu X. Gut microbiota modulation: a viable strategy to address medical needs in hepatocellular carcinoma and liver transplantation. *Engineering* 2023:online ahead of print. DOI
108. Shen YC, Lee PC, Kuo YL, et al. An exploratory study for the association of gut microbiome with efficacy of immune checkpoint inhibitor in patients with hepatocellular carcinoma. *J Hepatocell Carcinoma* 2021;8:809-22. DOI PubMed PMC
109. Mao J, Wang D, Long J, et al. Gut microbiome is associated with the clinical response to anti-PD-1 based immunotherapy in hepatobiliary cancers. *J Immunother Cancer* 2021;9:e003334. DOI PubMed
110. Sharma A, Roy A, Premkumar M, et al. Fecal microbiota transplantation in alcohol-associated acute-on-chronic liver failure: an openlabel clinical trial. *Hepatol Int* 2022;16:433-46. DOI
111. Huang HC, Tsai MH, Chang CC, et al. Microbiota transplants from feces or gut content attenuated portal hypertension and portosystemic collaterals in cirrhotic rats. *Clin Sci* 2021;135:2709-28. DOI