JHEP|Reports

The future of clinical trials of gut microbiome therapeutics in cirrhosis

Patricia P. Bloom[1,*], Raymond T. Chung[2,*]

Summary

The last two decades have witnessed an explosion of microbiome research, including in hepatology, with studies demonstrating altered microbial composition in liver disease. More recently, efforts have been made to understand the association of microbiome features with clinical outcomes and to develop therapeutics targeting the microbiome. While microbiome therapeutics hold much promise, their unique features pose certain challenges for the design and conduct of clinical trials. Herein, we will briefly review indications for microbiome therapeutics in cirrhosis, currently available microbiome therapeutics, and the biological pathways targeted by these therapies. We will then focus on the best practices and important considerations for clinical trials of gut microbiome therapeutics in cirrhosis.

Introduction

With the advent of modern gene sequencing techniques, there has been an explosion of microbiome research in the 21st century. This research has reached hepatology, first with studies demonstrating altered bacterial composition in liver disease, then expanding to other organisms like fungi and viruses, as well as to alterations in microbiome function. Most recently, efforts have been made to connect microbiome features to clinical outcomes. Today, microbiome research in hepatology is focused on the development of microbiome therapeutics.

Several recent reviews have expertly described our current understanding of the gut-liver axis, as well as potential microbiome therapeutics in liver disease.[1–12] Herein, we will briefly review indications for microbiome therapeutics in cirrhosis, currently available microbiome therapeutics, and the biological pathways targeted by these therapies. We will then focus on the best practices and important considerations for clinical trials of gut microbiome therapeutics in cirrhosis.

Indications for microbiome therapeutics in cirrhosis

Cirrhosis, regardless of aetiology, has been associated with a gut microbial community distinct from healthy controls.[13,14] Patients with cirrhosis exhibit depletion of *Faecalibacterium prausnitzii*, which has anti-inflammatory properties, and of several *Coprococcus*, *Lachnospiraceae*, and *Ruminococcaceae* species, which all produce short-chain fatty acids (SCFAs).[13] SCFAs are an important energy source for intestinal epithelia and therefore impact gut barrier function. Patients with cirrhosis have increased abundance of species of oral origin, and an increased number of genes that contribute to ammonia production.[13] Likely due to alterations in gut microbial composition, patients with cirrhosis also have elevated intestinal epithelial cell inflammation and a more permeable gut barrier.[15–17]

Particular complications of cirrhosis have also been linked to microbiome dysfunction and are thus potential indications for microbiome-targeted modulation. Table 1 summarises these indications as well as potentially targetable biological pathway(s). Potential future indications also include sarcopenia and improved quality of life metrics. Fig. 1 depicts the biological targets of most microbiome therapeutics in cirrhosis, including bile acid, SCFA, and ammonia metabolism, gut barrier function, and immune system constituents.

Types of microbiome therapeutics

There are several types of microbiome therapeutics, and several ways to select a microbiome therapeutic for a specific indication. Some microbiome therapeutics are derived through processing of human faeces, which can be purified and narrowed to different degrees (Fig. 2). Other microbiome therapeutics, like probiotics and prebiotics, are not derived directly from donor faeces. Table 2 summarises potential microbiome therapeutics in cirrhosis, including prebiotics, probiotics, synbiotics (combined prebiotic and probiotic), postbiotics, antibiotics, bacteriophages, antibodies to specific species, faecal microbiota transplant (FMT), and selected consortium products. Prebiotics are substrates selectively utilised by host microorganisms, conferring a health benefit.[26] Probiotics are living microorganisms that, when administered in adequate

* Corresponding authors. Addresses: 1500 E Medical Center Dr, Ann Arbor, MI 48109, USA; (P.P. Bloom), or Warren 1007, Liver Center, GI Division, Massachusetts General Hospital, Boston, MA 02114; (R.T. Chung).
E-mail addresses: ppbloom@med.umich.edu (P.P. Bloom), chung.raymond@mgh.harvard.edu (R.T. Chung).

https://doi.org/10.1016/j.jhepr.2024.101234

Keypoints

- There is no animal model for testing microbiome therapeutics that perfectly recapitulates human cirrhosis.
- There is much greater heterogeneity in how a patient with cirrhosis will respond to a microbiome therapeutic than a traditional pharmaceutical, which complicates the evaluation process.
- Validated surrogate endpoints, including patient-reported outcomes, will be needed to promote accelerated innovation and discovery in trials of microbiome therapeutics for cirrhosis.
- Striking a balance of inclusion criteria is essential; too many criteria will cripple enrolment and too few criteria will limit interpretation.
- Strain engraftment should be evaluated but may not be required for therapeutic success.
- Future microbiome therapeutic trials in cirrhosis should proactively monitor adverse events beyond gastrointestinal symptoms.

Table 1. Indications for microbiome therapeutics in cirrhosis.

Indication*	Possible biological mechanisms
Hepatic encephalopathy	• Depleted SCFA producers and SCFA levels[1,18] • Altered bile acid signalling increasing blood-brain-barrier permeability and neuroinflammation[19] • Increased abundance of *Streptococcus salivarius* and increased ammonia production[20]
Spontaneous bacterial peritonitis	• Bacteria-derived proteases degrade cell junction proteins of colonic epithelium[21]
Other infection	• Decreased species richness[14]
Decompensation	• Pathogen-associated molecular patterns translocate across the gut barrier via increased paracellular intestinal permeability, leading to systemic inflammation[7]
Antibiotic resistance	• Cirrhosis has a unique pattern of antimicrobial resistance genes, distinct from other chronic diseases[22,23]
Hepatocellular carcinoma	• Combination of low SCFA, increased intestinal permeability, and increased release of pathogen-associated molecular patterns[24]
Alcohol use disorder	• Systemic inflammation, microbial diversity, and SCFA production possibly linked to alcohol consumption[25]

SCFA, short-chain fatty acid.
*Often for prophylaxis against.

amounts, confer a health benefit on the host.[27] Postbiotics are defined as a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host, which can include SCFAs.[28] Bacteriophages are viruses that infect bacteria and can selectively lyse and eliminate certain bacterial species. Similarly, antibodies to specific microbial species can be developed, but the challenge here is that not all microbial pathology in cirrhosis can be linked to specific individual pathogenic strains. When we discuss microbiome therapeutics below, it could refer to any of the above therapies, unless otherwise specified.

Approaches to identify the optimal microbiome therapeutic include: 1) a "rational" approach – *i.e.*, identify the taxa or functional niche missing in cross-sectional studies, and find a therapeutic that contains those exact taxa or biological functions; 2) a bioinformatic approach – *i.e.*, use advanced statistical techniques to identify bacteria that would replace functions missing in the current microbiome; or 3) iteratively test different bacterial communities to identify a selected consortium product that yields the desired composition or functional result.

Clinical trial design considerations for microbiome therapeutics in cirrhosis

Clinical trial phases

Preclinical studies in animal models are the first step in testing most therapies (Fig. 3). There are many animal models available for the study of liver diseases, but three animal models of cirrhosis, in particular, are used in the majority of preclinical studies: carbon tetrachloride, thioacetamide, and common bile duct ligation.[42] These models have some strengths. For example, carbon tetrachloride can often produce cirrhosis and multiple manifestations of liver dysfunction, including hepatocyte apoptosis, ascites formation, and other forms of decompensation. Unfortunately, these models have several weaknesses. The carbon tetrachloride model varies from animal to animal, yielding a heterogeneous effect, and its withdrawal can improve fibrosis. Thioacetamide does not universally produce sufficient fibrosis to yield cirrhosis. Common bile duct ligation by design limits biliary flow, which limits the ability to comprehensively evaluate the clinical effect of microbiome therapeutics. Human cirrhosis develops from multiple aetiologies, and no single animal model perfectly reflects each aetiology of human cirrhosis. Not every animal model develops portal hypertension or hepatic encephalopathy with identical physiology to humans. Finally, and importantly for microbiome therapeutics, the gut microbiome differs between mice, rats, and humans.[43] Humans and mice share 89% of the same bacterial genera, but the abundance of each of these bacteria vary substantially between the two species.[44]

Given the limitations of animal models for testing microbiome therapeutics in cirrhosis, this traditional phase of testing does not have the same high degree of utility as it does for other therapeutics and disease states. Humanised animal models containing stool from a human donor with cirrhosis, or a bioreactor, could be used to evaluate if living components of a microbiome therapeutic integrate into the indigenous community or impact the immune and metabolic functions of the microbiome. However, these models will not perfectly recapitulate human physiology and are unlikely to be useful for evaluating clinical outcomes. As an example, a genetically engineered *Escherichia coli Nissle* designed to convert ammonia to arginine was used successfully to treat hyperammonaemia in a mouse model.[45] However, this product failed to lower serum ammonia in healthy human controls as well as humans with cirrhosis.[45,46] The reason behind these discrepant

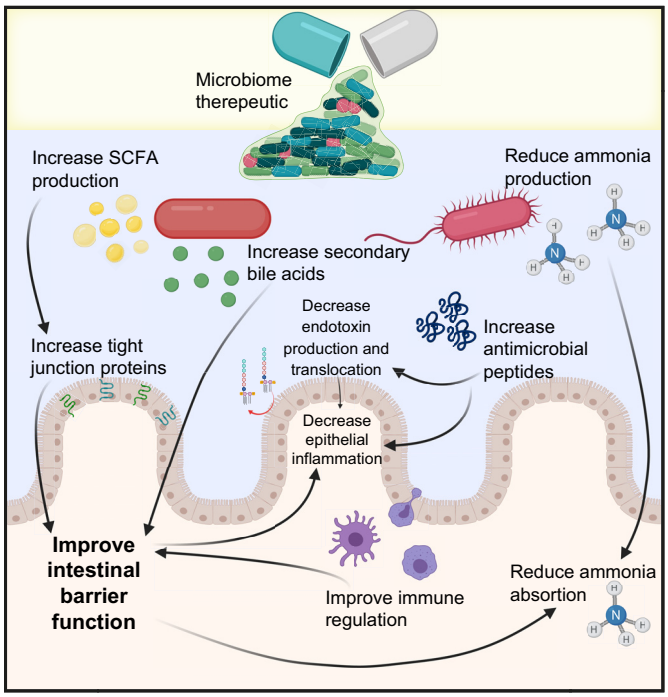

Fig. 1. Biological targets of microbiome therapeutics in cirrhosis. Microbiome therapeutics have multiple potential mechanisms in cirrhosis, including several which could improve intestinal barrier function including increasing SCFA, secondary bile acid, tight junction protein, and antimicrobial peptide production. SCFAs are a primary energy source for colonic enterocytes, which allow them to produce tight junctions and mucin, thus bolstering the epithelial barrier. Patients with cirrhosis also have elevated intestinal epithelial cell inflammation and a more permeable gut barrier. By changing microbiome composition and function, ammonia and endotoxin production and translocation could decrease. Adapted from published figure.[1] SCFA, short-chain fatty acid.

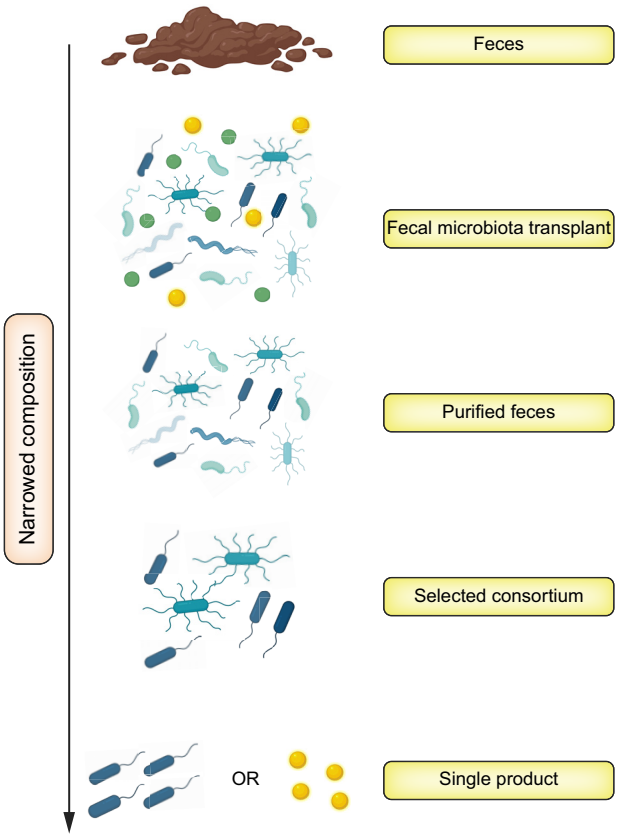

Fig. 2. Ranging compositions of microbiome therapeutics derived from human faeces. A range of microbiome therapeutics can be derived from human faeces. The therapeutics range from broad to more narrow compositions. From faeces to FMT, a cryoprotectant should be added and the material should be frozen at -80 °C until use.[102] Faeces can be further purified by various solvents to remove bacterial, viral, fungal, and parasitic material. Broader compositions may be more complete and therefore better able to stably colonise. However, narrow compositions may provide a targeted effect with less chance of off-target effects or pathogen transmission. FMT, faecal microbiota transplant.

results is not entirely clear – perhaps because the animal models did not contain human indigenous bacterial communities, or because of species differences in gut barrier or hepatic physiology. A recent study of bacteriophages targeting cytolysin-producing *Enterococcus faecalis* showed marked success in treating alcohol-induced liver injury in humanised mice.[47] Human trials are now underway to see if this particular therapy can make the leap from animals to humans.[48]

Traditional phase I studies in healthy controls may similarly not have sufficient utility for cirrhosis-directed microbiome therapeutics. Phase I studies typically include 20 to 100 healthy volunteers or people with the condition of interest.[49] The objectives are to evaluate safety and optimal dosing. The challenge in trials of microbiome therapeutics is that healthy individuals have different gut microbiome composition and function than patients with cirrhosis.[13,14] Given differences in baseline microbiome composition, it is possible that a microbiome therapeutic will have a substantially different effect in a healthy person than a patient with cirrhosis.

Given the limited value of preclinical and healthy volunteer studies as described above, in most cases it is most useful to start the evaluation of cirrhosis-directed microbiome therapeutics in small phase I-II studies of patients with cirrhosis. The aim of these studies is to evaluate colonisation of microbiome therapeutic components, effects on recipient microbiome function, safety, and optimal dosing.

Phase II studies typically enrol several hundred patients with the condition. The objectives are to determine efficacy and side effects. Recruiting a large and relatively homogeneous population of patients with cirrhosis requires numerous study sites, precise coordination, and therefore substantial funding. The particular benefit of these larger studies in microbiome therapeutic trials is being able to evaluate the colonisation and clinical efficacy in patient subgroups of varying cirrhosis aetiology, indigenous bacterial composition, and concomitant medication use. For example, in one study of FMT to treat hepatic encephalopathy, it was found that baseline recipient *Bifidobacterium* abundance may have influenced clinical outcomes.[40] Larger phase II studies will allow for better evaluation of possible variable efficacy in subgroups.

Phase III studies typically include hundreds to thousands of patients with the condition. Enrolment of this many patients is a challenge in cirrhosis, requiring large and almost always international consortia. Diets and microbiome composition vary regionally, resulting in further heterogeneity of effect and an increased sample size requirement to reach adequate statistical power.[50,51] Despite the challenge of recruiting a larger

Table 2. Microbiome therapeutics in cirrhosis.

Microbiome therapeutic	Notable trials or meta-analyses in cirrhosis	Summary of clinical outcomes	Mechanism
Prebiotic	• Two large meta-analyses comparing non-absorbable disaccharides to placebo and other options to treat HE[29,30]	• Lactulose reverses minimal HE, prevents overt HE, and improves quality of life	• Lactulose increases beneficial taxa, SCFA production, inhibits pathogen growth[11]
Probiotic	• Two large meta-analyses of probiotics to treat HE[30,31]	• Probiotics improve HE symptoms, reverse minimal HE, prevent overt HE; however, trials are low to moderate quality and at high risk of bias	• Improve intestinal barrier function, immune modulation, decrease portal hypertension[1]
Synbiotic	• Single-centre trial of *Bifidobacterium longum* and fructo-oligosaccharide[32] • Single-centre trial of synbiotics compared to placebo or prebiotic alone[33]	• Cognitive benefit with synbiotics, but no clear superiority to prebiotics alone in this limited data set	• Similar to prebiotics and probiotics above
Postbiotic	• No published clinical trials in humans yet	• n.a.	• In theory, improved intestinal barrier function
Antibiotic	• Multicentre randomised-controlled trial of rifaximin to prevent future HE[34] • Multicentre randomised trial of three antibiotics to treat SBP[35]	• Rifaximin reduces the recurrence of overt HE • Cefotaxime, ceftriaxone, and ciprofloxacin were equally effective in treating SBP	• Rifaximin reduces mucin-degrading species, modulates intestinal immune microenvironment, and reduces endotoxin translocation[36,37]
Bacteriophage	• No published clinical trials in humans yet	• n.a.	• In theory, selective elimination of deleterious bacterial strains
Antibodies to bacteria	• No published clinical trials in humans yet	• n.a.	• In theory, selective elimination of deleterious bacterial strains
FMT	• Pilot trials of FMT to treat HE[38–40] • Pilot trial of FMT to treat alcohol use disorder[25]	• Improved cognitive tests, largely safe • Decreased alcohol cravings and urine biomarkers	• Increases SCFA, secondary bile acid, tight junction protein, and antimicrobial peptide production • Ammonia and endotoxin production decreases
Selected Consortium Product	• Pilot trial of VE303 (8 clostridial strains) to treat HE[41]	• No significant safety difference between VE303 and placebo • 67% VE303 patients improved cognitive scores, compared to 33% with placebo	• Not yet published

FMT, faecal microbiota transplant; HE, hepatic encephalopathy; SBP, spontaneous bacterial peritonitis; SCFA, short-chain fatty acid.

patient cohort, these larger studies allow for further evaluation of microbiome therapeutics in disparate patient subgroups and baseline enterotypes.

Clinical trial endpoints

Endpoint selection is critical for the design of any trial. Endpoint selection influences the design of the trial and the conclusions drawn. These endpoints then determine the regulatory outcomes for those therapies.

Appropriate clinical trial endpoints in cirrhosis have recently been reviewed by the LiverHope Consortium, and therefore will not be exhaustively reviewed here.[52] The principal accepted endpoints include survival (or transplant-free survival), hospitalisations, and new or worsening decompensation. The ideal endpoints depend on clinical trial phase, as described above.

Health-related quality of life is poor in cirrhosis, especially decompensated cirrhosis.[53] While traditional primary endpoints such as hospitalisation or survival are of course critical, quality of life is vitally important to our patients. There are several well-

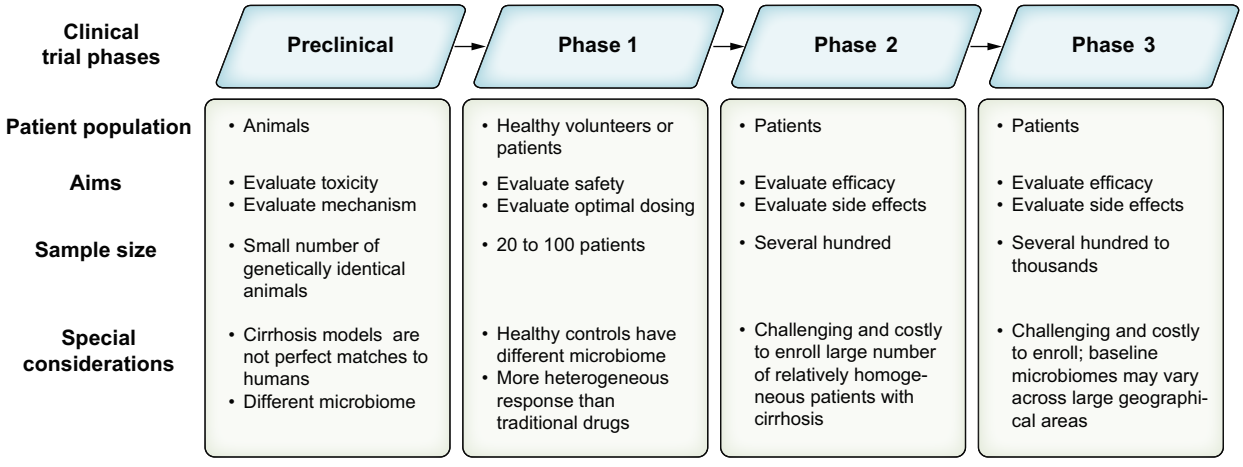

Fig. 3. Phases of clinical trials for microbiome therapeutics in cirrhosis. Patient population, aims, sample size and special considerations at each clinical trial phase for microbiome therapeutics in cirrhosis.

validated patient-reported outcomes in cirrhosis, though these have not yet been accepted by the FDA or the EMA as validated primary endpoints.[54] Recent pilot trials have been conducted with patient-reported outcomes, including muscle cramps and health-related quality of life, as primary outcomes.[55,56] Further validation of some patient-reported outcomes and advocacy (to encourage regulatory bodies to accept them as clinical endpoints) are needed prior to their adoption as primary endpoints in trials.

Furthermore, unlike many other chronic diseases, cirrhosis lacks many validated surrogate endpoints. For example, blood pressure has been shown to predict mortality from cardiovascular disease, and therefore can be employed as a surrogate endpoint in cardiovascular disease trials. Very few analogous validated proxies exist in cirrhosis, but development and validation of these proximal endpoints would ease enrolment requirements and potentially promote accelerated innovation and discovery. Interestingly, several noteworthy microbiome therapeutic trials in cirrhosis have not used the stringent trial endpoints outlined by the LiverHope Consortium above. For example, the phase III, multicentre, randomised-controlled trial of rifaximin to prevent hepatic encephalopathy used "an increase from a baseline Conn score of 0 or 1 to a score of 2 or more or from a baseline Conn score of 0 to a Conn score of 1 plus a 1-unit increase in the asterixis grade" to define their primary outcome of breakthrough hepatic encephalopathy, rather than hospitalisation for hepatic encephalopathy.[34]

Study design

Phase II and III trials should be randomised, blinded, and placebo-controlled to minimise selection bias, observer bias, and response bias. These potential biases need to be minimised to adequately evaluate efficacy. However, phase I trials can be open-label and still identify safety concerns and discern optimal dosing. Dose finding is more complex with microbiome therapeutics than with traditional pharmaceuticals. First, microbiome therapeutics often contain multiple components (*e.g*. prebiotics combined with multiple bacterial strains), so the optimal ratios as well as the absolute number of colony-forming units require evaluation. Second, pre-treatment antibiotics or bowel preparation will possibly influence engraftment of the microbiome therapeutic, and therefore combinations of these pre-treatments must be tested.

Early-stage trials of microbiome therapeutics should be designed for rapid cycling of different doses and combinations of constituents to allow for timely discovery of the optimal consortium product. As an example, VE303 is a selected consortium of eight clostridial strains designed to treat refractory and recurrent *C. difficile* infection. In a phase I trial of 39 healthy volunteers, the study investigators divided the group into nine cohorts and trialled different dose regimens and pre-treatment strategies.[57] They found that a particular multi-day dosing of VE303 after vancomycin pre-treatment yielded consistent strain engraftment for up to 1 year. Adaptive trials such as this allow for rapid iterative changes to trial design or intervention between sub-cohorts, allowing for efficiency in dose finding and optimisation of pre-treatment regimens.[58,59] A platform trial is another study design that would be useful for efficient evaluation of multiple microbiome therapeutics. Platform trials allow for the study of multiple therapies simultaneously, allowing therapies to enter or leave the platform based on their success per a pre-established decision algorithm.[60] In this design, patients are also stratified by certain baseline biomarkers, which in this case could be baseline recipient microbiome features. It is not currently clear which biomarkers should be used, and a recent Delphi consensus highlighted the analytic and reproducibility challenges in developing such biomarkers.[61]

Microbiome therapeutic delivery in the patient with cirrhosis must be considered in the context of other cirrhosis-specific therapies (beta blockers, statins), including several which are known to influence the microbiome (lactulose, rifaximin, prophylactic antibiotics). Sequential multiple assignment randomised trials (SMARTs) involve randomising patients at multiple sequential decision points, mimicking the natural history of clinical decision making. SMARTs would allow for greater understanding of how these microbiome therapeutics fit into the larger context of other therapies for cirrhosis and allow for the development of validated treatment pathways.

The specific microbiome therapeutic and stage of testing will influence the selection of study design. At the earliest stage, an adaptive trial design may be ideal to efficiently identify the ideal dose and pre-treatment regimen (if needed). At later stages, SMARTs can be used to understand the ideal timing or sequencing of microbiome therapeutics with other cirrhosis therapies. Finally, a platform or a traditional stratified randomised-controlled trial could be used to understand if there are important biomarkers that influence the efficacy of certain microbiome therapeutics.

Population selection

Patients with cirrhosis are a heterogeneous group, which complicates patient selection and ultimately data interpretation (Table 3). The factors that vary across patients with cirrhosis can influence a) risk of developing the primary outcome; b) engraftment and functional output of the microbiome therapeutic; and c) risk of adverse events caused by the microbiome therapeutic. Therefore, inclusion and exclusion criteria are critical to study design.

First, a patient's cirrhosis can be compensated or decompensated. Decompensated cirrhosis is characterised by the presence of ascites, hepatic encephalopathy, or portal hypertensive bleeding. Patients with decompensated cirrhosis are more likely than patients with compensated cirrhosis to experience typical clinical trial endpoints including new or worsening decompensation, hospitalisation, and death. Patients with cirrhosis and portal hypertension are also at greater risk of those endpoints than patients without portal hypertension. Portal hypertension and the presence of ascites may also increase the risk of adverse events with certain microbiome therapeutics, though this is unproven. Patients with portal hypertension, including ascites, are at greater risk of bacterial translocation – thus there is a potential risk of translocation of some of the bacteria contained in the microbiome therapeutic.[68] However, this risk may be largely theoretical as many microbiome therapeutics contain bacteria that have a net beneficial effect on gut barrier function.

Polypharmacy is common in patients with cirrhosis, including use of opiates, benzodiazepines, proton pump

Table 3. Potential enrolment criteria for trials of microbiome therapeutics in cirrhosis.

Characteristic	How to evaluate	Considerations
Decompensation	• Ascites, HE, or portal hypertensive bleeding • May include patients well-managed with medical therapy • Re-compensated patients may differ from decompensated and other compensated patients	• Will patients with no ascites on diuretics be included? • Will prior overt HE qualify if the last episode was >1 year ago? • Who can make the diagnosis of decompensation (research coordinator, medical doctor, hepatologist)?
Portal hypertension	• Transjugular assessment of hepatic venous pressure gradient has been the traditional gold standard[62] • Increasingly transient elastography, platelet count, or presence of ascites or varices are an acceptable non-invasive method	• Transient elastography is less specific for portal hypertension in obesity
Aetiology of cirrhosis	• Most often based on gastroenterologist or hepatologist assessment	• Patients labelled as having MASLD may also have under-reported harmful alcohol consumption[63]
Alcohol use	• Biomarkers such as phosphatidylethanol or ethyl glucuronide can aid in diagnosis[63,64]	• Alcohol influences microbiome composition and function[65]
Polypharmacy	• Detailed medication history at screening, including for proton pump inhibitors, antibiotics, opiates, benzodiazepines, lactulose, rifaximin, beta blockers, and statins	• Avoiding all of these will severely limit enrolment; be selective about which should be exclusion criteria or stratification criteria
Diet	• Could have a registered dietician perform a 24-hour dietary recall on two occasions • The Nutrition Data System for Research is well validated[66]	• Diet varies for most individuals, so additional dietary recalls increase comprehensiveness
MELD 3.0	• Use published calculation[67]	• MELD does not perfectly predict mortality, especially in some cases, including hepatopulmonary syndrome or refractory hepatic hydrothorax

HE, hepatic encephalopathy; MASLD, metabolic dysfunction-associated steatotic liver disease; MELD, model for end-stage liver disease.

inhibitors, and antibiotics.[69] Opiates and benzodiazepines increase the risk of hepatic encephalopathy.[70] Proton pump inhibitors influence gut microbiome composition by decreasing diversity, increasing oral flora in the colon, and increasing the abundance of potentially pathogenic strains.[71,72] Many patients with cirrhosis are on antibiotics for primary or secondary prophylaxis of spontaneous bacterial peritonitis, or active treatment of infections. Fluoroquinolones, a common class of such prophylactic antibiotics, influence the gut microbiome by decreasing alpha diversity and *Lachnospiraceae* and *Ruminococcaceae* genera, which are found in many probiotic bacterial strains.[73] Finally, as has been reviewed in detail elsewhere, lactulose and rifaximin influence gut microbiome composition and thus are likely to modify the effect of microbiome therapeutics.[1,11,12]

It is well-documented that diet influences microbiome content and function. Amongst patients with cirrhosis, diets enriched in fermented milk, yogurt, vegetables, cereals, coffee and tea were linked to higher microbial diversity.[50,51] Fermented foods such as kombucha, yogurt, and kimchi increase microbial diversity and decrease inflammatory markers.[74] Finally, resistant starch can be utilised by certain enteric bacteria to elicit large increases in butyrate and acetate production.[75,76] Despite the effect of diet on gut microbiome composition and function, diet has not historically been restricted in cirrhosis trials. In fact, dietary interventions are ripe for clinical trial investigation in cirrhosis. Many patients prefer dietary recommendations to medications, and a few small diet intervention studies have been feasible in cirrhosis.[77,78] According to ClinicalTrials.gov, several dietary intervention trials in cirrhosis are actively recruiting (NCT03080129, NCT06328088, NCT06425380).

Including the complete set of characteristics described in Table 3 as enrolment criteria would severely impair feasibility. Constraining the patient population too narrowly can lead to poor enrolment and poor external validity. If the trial cannot be feasibly completed because of overly restrictive criteria, it will never yield results and will not advance scientific knowledge. In addition, if trial enrolment is overly restrictive, the results will not be broadly applicable to the larger population of patients with cirrhosis. Maintaining external validity is critical to yielding real-world impact from clinical trials. Thus, when designing a clinical trial of microbiome therapeutics in cirrhosis, investigators must select enrolment criteria judiciously. The degree of cirrhosis severity by some measure (compensated *vs.* decompensated, portal hypertension *vs.* not, MELD 3.0 cut-off) should almost certainly be included as this is such a significant predictor of important clinical outcomes. The remaining criteria should be selected carefully based on the biological mechanism of the microbiome therapeutic and the primary outcome of interest. Furthermore, a limited number of stratification variables can be employed for important covariates not used as enrolment criteria; this number depends on the projected sample size of the trial.

Sample size determinations

Sample size calculations in trials of microbiome therapeutics in patients with cirrhosis will depend on a) the heterogeneity of the microbiome therapeutic; b) the heterogeneity of the patient population; and c) the selected primary outcome. Some microbiome therapeutics are clonally identical from batch to batch, but others, like FMT, vary from lot to lot. While FMT appears to treat recurrent and refractory *C. difficile* colitis without batch or donor effect, this is likely not the case for other conditions.[79] This heterogeneity in product is likely to result in heterogeneity of treatment effect in patients with cirrhosis. As discussed previously, more restrictive exclusion criteria will yield a more homogeneous patient population, likely to have a more consistent response to therapy. However, this comes at the cost of slower enrolment. In addition, the recipient microbiome likely modifies or influences the effect of microbiome therapeutics.[80] Microbiome therapeutics are not being administered into a vacuum; rather, they are introduced into an existing environment. In fact, the human gut microbiome harbours more inter-person variability than other components of

the human drug metabolism machinery. There are 98 well-characterised variants of the eight cytochrome p450 drug-metabolizing enzymes, whereas the gut microbiome has far greater genetic and phenotypic diversity.[81,82] Therefore, depending on the microbiome therapeutic, there may be more heterogeneous responses than with other drugs, requiring larger sample sizes to achieve adequate power.

As described above, there are many patient characteristics which may act as effect modifiers for microbiome therapeutics. Common examples in cirrhosis include use of proton pump inhibitors, rifaximin, or the aetiology of liver disease. Investigators may consider including these as stratifying variables at enrolment, ensuring there are large enough samples in each sub-group to answer questions about the impact of that factor. In addition, if recipients' baseline microbiome is found to be a significant effect modifier, future trials should consider conducting quick stool PCR or functional assays at screening to identify key baseline bacteria or metabolites and to enable stratification by those baseline factors. The impact of stratification on sample size depends on several factors but tends not to have a large impact on sample size requirements in superiority trials (under which most microbiome therapeutic trials for cirrhosis fall).[83]

Regulatory considerations

Microbiome therapeutics carry unique regulatory challenges. According to the FDA, an active ingredient is "any component that is intended to furnish pharmacological activity… in the… cure… of disease."[84] In some microbiome therapeutics, like FMT, the precise active ingredient(s) is not known. FMT is derived from a human donor and therefore varies from lot to lot. Without a clearly defined active ingredient, it is impossible to know if the active ingredient is consistent in each dose.

Another regulatory consideration for microbiome therapeutics is whether the therapy will be viewed as a biological agent, human tissue product, or medicinal product. Each of these categories are regulated differently within each country, but also the designations and regulations vary from one governing body to another. A review by Merrick *et al.* summarises the myriad designations of FMT across the world, and how these designations have impacted its regulation.[85]

Microbiome therapeutics derived from human donor faeces carry another important regulatory consideration: an inherent risk of transmitting infection. In the vast majority of cases, FMT has safely treated *C. difficile* infection; however, it is still possible to transmit pathogenic organisms via FMT even when following FDA-approved screening protocols.[86–88] There will always be unknown pathogens, or pathogens that are challenging to detect by testing. The sensitivity of stool tests to detect pathogens present in low abundance is currently limited.

Finally, when microbiome therapeutics contain living bacteria, the manufacturer must ensure that those bacteria stay alive, durably, until they arrive in the intestinal lumen of the patient. Ensuring bacterial viability during production, storage, administration, and enteric transit is a challenge at every stage.

Importance of strain engraftment

For microbiome therapeutics that contain bacteria, it is possible to assess strain engraftment through metagenomic sequencing of the microbiome therapy and the patient's stool samples before and after therapy, followed by analysis of these samples with one of several validated engraftment analysis platforms.[89,90] There are analytical challenges with summarising engraftment across a study when the product (such as FMT) differs between patients.

Strain engraftment of microbiome therapeutics has been linked to desired clinical outcomes in some trials, but not all. In a trial of SER-109 to treat recurrent and refractory *C. difficile* infection, engraftment was linked to cure.[91] However, in a trial of FMT for advanced melanoma, clinical responders and non-responders had similar rates of FMT strain engraftment.[92] Strain engraftment could lead to a functional change in microbiome function, which then leads to the desired clinical outcome. However, engraftment may not be necessary for therapeutic effect. Further complicating the picture, it is possible that the underlying dysbiosis of cirrhosis impedes engraftment of FMT. One study found that patients with cirrhosis required additional doses of FMT to cure their *C. difficile* infection, potentially due to challenges with engraftment.[93]

For microbiome therapeutics that contain bacteria, it is important for clinical trials to determine the degree and durability of strain engraftment for two primary reasons: 1) to understand the therapy's mechanism of action; and 2) to understand if engraftment of therapy strains into the recipient is correlated with desired clinical outcomes. Further complicating the question of engraftment, it is possible that mucosal strains differ from stool strains and in some cases mucosal microbiota may be more metabolically and immunologically active.[16,94] While it would be ideal to sample both compartments, this would not be feasible in larger clinical trials.

Clinical trials should be designed to understand how long-lasting strain engraftment needs to be to elicit a sustained therapeutic outcome. Also, it would be useful to know if there is a certain abundance cut-off or other functional microbiome outcome (for example, faecal butyrate levels) associated with clinical success. If identified, these endpoints could become surrogate biomarkers of efficacy for future trials.

Antimicrobial resistance

There is a high incidence of antimicrobial resistance in patients with cirrhosis.[95] The incidence of antimicrobial resistance increases with disease severity and decompensation, and is associated with poor outcomes including hospitalisation and mortality.[23] It is therefore important that microbiome therapeutics for patients with cirrhosis do not increase the burden of antimicrobial resistance genes, and ideally decrease this burden. There have been several cases of multidrug-resistant organisms transmitted through FMT, including to patients with cirrhosis, though this is rare with current screening procedures.[86,87] In two small trials of FMT in cirrhosis, antimicrobial resistance genes were largely decreased, though notably some resistance genes increased.[22] In a larger study outside of the cirrhosis field, FMT led to a long-term decrease in antimicrobial resistance.[96] Even outside of FMT, it is important for microbiome therapeutic trials in cirrhosis to evaluate and report their impact on antimicrobial resistance.

Safety monitoring

Nearly two decades since the launch of the National Institutes of Health Human Microbiome Project, there is abundant data

connecting the gut microbiome to nearly every human organ system. Microbiome therapeutics have been shown to impact gastroenterological, neurological, oncologic, and immunological conditions, amongst others.[97–100] Therefore, it is possible that microbiome therapeutics have an impact, favourable or unfavourable, outside the gastrointestinal tract and liver.

Microbiome therapeutic trials in liver disease have largely reported gastrointestinal side effects, but that may relate to bias in the adverse event assessment process. It is not clear that trials are recording weight, immune or inflammatory events, skin condition changes, mood changes, and many more possible adverse events. It is up to the site investigator to determine if a symptom constitutes an adverse event and to determine its relatedness.

Future microbiome therapeutic trials in cirrhosis should proactively monitor adverse events beyond gastrointestinal symptoms. Ultimately, data from multiple trials should be pooled to evaluate possible uncommon and linked adverse events. Finally, to date, microbiome therapy trials in cirrhosis have included brief follow-up periods (1 year at most). In the future we need to better understand the possible long-term effects of microbiome manipulation.[101]

Conclusion

Mounting evidence has shown that patients with cirrhosis have altered gut microbiome composition and function compared to several control populations. Furthermore, it is clear that gut metabolism and immune response impact important clinical outcomes in cirrhosis. The hepatology community has now entered the era of modulating the gut microbiome for therapeutic benefit. As this review has laid out, there are important and unique considerations for clinical trials of microbiome therapeutics in patients with cirrhosis. Navigating these nuances is feasible and will be key to realising the full potential of microbiome therapeutics in this population.

Affiliations

[1]*University of Michigan, Division of Gastroenterology, Ann Arbor, MI, USA;* [2]*Massachusetts General Hospital, Division of Gastroenterology, Boston, MA, USA*

Abbreviations

FMT, faecal microbiota transplant; SCFA, short-chain fatty acid; SMARTs, sequential multiple assignment randomised trials.

Financial support

None for this project.

Conflict of interest

PPB: Research grant from Vedanta Biosciences, consulting with Nexilico; RTC: Research grants from Synlogic, Kaleido.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

PPB: collecting and reviewing literature, drafting the manuscript, figure and table creation; RTC: planning of the study, critical revisions of the manuscript. All authors approved the final version of the manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2024.101234.

References

Author names in bold designate shared co-first authorship

[1] Bloom P, Tapper EB, Young VB, et al. Microbiome therapeutics for hepatic encephalopathy. J Hepatol 2021. https://doi.org/10.1016/j.jhep.2021.08.004. Epub 20210825. PubMed PMID: 34453966.

[2] Caraceni P, Vargas V, Solà E, et al. The use of rifaximin in patients with cirrhosis. Hepatology 2021. https://doi.org/10.1002/hep.31708. Epub 2021/01/10. PubMed PMID: 33421158.

[3] Acharya C, Bajaj JS. Altered microbiome in patients with cirrhosis and complications. Clin Gastroenterol Hepatol 2019;17(2):307–321. https://doi.org/10.1016/j.cgh.2018.08.008. Epub 20180809. PubMed PMID: 30099098; PubMed Central PMCID: PMC6314917.

[4] Chopyk DM, Grakoui A. Contribution of the intestinal microbiome and gut barrier to hepatic disorders. Gastroenterology 2020;159(3):849–863. https://doi.org/10.1053/j.gastro.2020.04.077. Epub 2020/06/23. PubMed PMID: 32569766; PubMed Central PMCID: PMC7502510.

[5] Tripathi A, Debelius J, Brenner DA, et al. The gut-liver axis and the intersection with the microbiome. Nat Rev Gastroenterol Hepatol 2018;15(7):397–411. https://doi.org/10.1038/s41575-018-0011-z. Epub 2018/05/12. PubMed PMID: 29748586; PubMed Central PMCID: PMC6319369.

[6] Tilg H, Adolph TE, Trauner M. Gut-liver axis: pathophysiological concepts and clinical implications. Cell Metab 2022;34(11):1700–1718. https://doi.org/10.1016/j.cmet.2022.09.017. Epub 20221007. PubMed PMID: 36208625.

[7] Trebicka J, Macnaughtan J, Schnabl B, et al. The microbiota in cirrhosis and its role in hepatic decompensation. J Hepatol 2021;75(Suppl 1):S67–s81. https://doi.org/10.1016/j.jhep.2020.11.013. PubMed PMID: 34039493; PubMed Central PMCID: PMC8973011.

[8] Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: pathophysiological basis for therapy. J Hepatol 2020;72(3):558–577. https://doi.org/10.1016/j.jhep.2019.10.003. Epub 2019/10/18. PubMed PMID: 31622696.

[9] Bajaj JS, Ng SC, Schnabl B. Promises of microbiome-based therapies. J Hepatol 2022;76(6):1379–1391. https://doi.org/10.1016/j.jhep.2021.12.003. PubMed PMID: 35589257; PubMed Central PMCID: PMC9588437.

[10] Acharya C, Bajaj JS. Chronic liver diseases and the microbiome-translating our knowledge of gut microbiota to management of chronic liver disease. Gastroenterology 2021;160(2):556–572. https://doi.org/10.1053/j.gastro.2020.10.056. Epub 2020/12/01. PubMed PMID: 33253686.

[11] Bloom PP, Tapper EB. Lactulose in cirrhosis: current understanding of efficacy, mechanism, and practical considerations. Hepatol Commun 2023;7(11). https://doi.org/10.1097/hc9.0000000000000295. Epub 20231012. PubMed PMID: 37820287; PubMed Central PMCID: PMC10578757.

[12] Bloom PP, Bajaj JS. The current and future state of microbiome therapeutics in liver disease. Am J Gastroenterol 2024;119(1s):S36–s41. https://doi.org/10.14309/ajg.0000000000002581. PubMed PMID: 38153225.

[13] Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature 2014;513(7516):59–64. PubMed PMID: 77.

[14] Solé C, Guilly S, Da Silva K, et al. Alterations in gut microbiome in cirrhosis as assessed by quantitative metagenomics: relationship with acute-on-chronic liver failure and prognosis. Gastroenterology 2021;160(1):206–218.e13. https://doi.org/10.1053/j.gastro.2020.08.054. Epub 20200914. PubMed PMID: 32941879.

[15] Riva A, Gray EH, Azarian S, et al. Faecal cytokine profiling as a marker of intestinal inflammation in acutely decompensated cirrhosis. JHEP Rep 2020;2(6):100151. https://doi.org/10.1016/j.jhepr.2020.100151. Epub 20200730. PubMed PMID: 32838247; PubMed Central PMCID: PMC7391986.

[16] Bloom PP, Bassis CM, Nojkov B, et al. Regional changes in intestinal permeability in cirrhosis are associated with mucosal bacteria. Hepatol Commun 2023 Sep 27;7(10):e0221.

[17] Bloom PP, Rao K, Bassis CM, et al. Duodenal permeability is associated with mucosal microbiota in compensated cirrhosis. Clin Transl Gastroenterol 2022;13(10):e00522. https://doi.org/10.14309/ctg.0000000000000522. Epub 20221001. PubMed PMID: 36000993; PubMed Central PMCID: PMC9624490.

[18] Bloom PP, Luevano JM, Miller KJ, et al. Deep stool microbiome analysis in cirrhosis reveals an association between short-chain fatty acids and hepatic encephalopathy. Ann Hepatol 2021 Nov-Dec;25:100333.

[19] DeMorrow S. Bile acids in hepatic encephalopathy. J Clin Exp Hepatol 2019;9(1):117–124. https://doi.org/10.1016/j.jceh.2018.04.011. Epub 20180504. PubMed PMID: 30774268; PubMed Central PMCID: PMC6363980.

[20] Zhang Z, Zhai H, Geng J, et al. Large-scale survey of gut microbiota associated with MHE via 16S rRNA-based pyrosequencing. Am J Gastroenterol 2013;108(10):1601–1611. PubMed PMID: 67.

[21] Haderer M, Neubert P, Rinner E, et al. Novel pathomechanism for spontaneous bacterial peritonitis: disruption of cell junctions by cellular and bacterial proteases. Gut 2022;71(3):580–592. https://doi.org/10.1136/gutjnl-2020-321663. Epub 20210311. PubMed PMID: 33707230; PubMed Central PMCID: PMC8862089.

[22] Bajaj JS, Shamsaddini A, Fagan A, et al. Fecal microbiota transplant in cirrhosis reduces gut microbial antibiotic resistance genes: analysis of two trials. Hepatol Commun 2021;5(2):258–271. https://doi.org/10.1002/hep4.1639. Epub 2021/02/09. PubMed PMID: 33553973; PubMed Central PMCID: PMC7850310.

[23] Shamsaddini A, Gillevet PM, Acharya C, et al. Impact of antibiotic resistance genes in gut microbiome of patients with cirrhosis. Gastroenterology 2021;161(2):508–521.e7. https://doi.org/10.1053/j.gastro.2021.04.013. Epub 20210420. PubMed PMID: 33857456; PubMed Central PMCID: PMC9069394.

[24] Schwabe RF, Greten TF. Gut microbiome in HCC - mechanisms, diagnosis and therapy. J Hepatol 2020;72(2):230–238. https://doi.org/10.1016/j.jhep.2019.08.016. PubMed PMID: 31954488.

[25] Bajaj JS, Gavis EA, Fagan A, et al. A randomized clinical trial of fecal microbiota transplant for alcohol use disorder. Hepatology 2020. https://doi.org/10.1002/hep.31496. Epub 2020/08/05. PubMed PMID: 32750174.

[26] Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 2017;14(8):491–502. https://doi.org/10.1038/nrgastro.2017.75. Epub 2017/06/15. PubMed PMID: 28611480.

[27] Hill C, Guarner F, Reid G, 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(8):506–514. https://doi.org/10.1038/nrgastro.2014.66. Epub 2014/06/11. PubMed PMID: 24912386.

[28] Salminen S, Collado MC, Endo A, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat Rev Gastroenterol Hepatol 2021;18(9):649–667. https://doi.org/10.1038/s41575-021-00440-6. Epub 20210504. PubMed PMID: 33948025; PubMed Central PMCID: PMC8387231.

[29] Gluud LL, Vilstrup H, Morgan MY. Non-absorbable disaccharides versus placebo/no intervention and lactulose versus lactitol for the prevention and treatment of hepatic encephalopathy in people with cirrhosis. Cochrane Database Syst Rev 2016;2016(5):Cd003044. https://doi.org/10.1002/14651858.CD003044.pub4. Epub 2016/05/07. PubMed PMID: 27153247; PubMed Central PMCID: PMC7004252 sponsored by Norgine. All review authors have conducted previous reviews on hepatic encephalopathy and two authors (Hendrik Vilstrup and Marsha Morgan) have conducted RCTs on hepatic encephalopathy. These previous research activities are an academic bias based on the definitions given in the Cochrane Hepato-Biliary Group module.

[30] Dhiman RK, Thumburu KK, Verma N, et al. Comparative efficacy of treatment options for minimal hepatic encephalopathy: a systematic review and network meta-analysis. Clin Gastroenterol Hepatol 2020;18(4):800–812.e25. https://doi.org/10.1016/j.cgh.2019.08.047. Epub 20190830. PubMed PMID: 31476436.

[31] Dalal R, McGee RG, Riordan SM, et al. Probiotics for people with hepatic encephalopathy. Cochrane database Syst Rev 2017;2:CD008716. PubMed PMID: 93.

[32] Malaguarnera M, Greco F, Barone G, et al. Bifidobacterium longum with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. Dig Dis Sci 2007;52(11):3259–3265. https://doi.org/10.1007/s10620-006-9687-y. Epub 2007/03/30. PubMed PMID: 17393330.

[33] Liu Q, Duan ZP, Ha DK, et al. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. Hepatology 2004;39(5):1441–1449. https://doi.org/10.1002/hep.20194. Epub 2004/05/04. PubMed PMID: 15122774.

[34] Bass NM, Mullen KD, Sanyal A, et al. Rifaximin treatment in hepatic encephalopathy. New Engl J Med 2010;362(12):1071–1081. PubMed PMID: 54.

[35] Yim HJ, Kim TH, Suh SJ, et al. Response-guided therapy with cefotaxime, ceftriaxone, or ciprofloxacin for spontaneous bacterial peritonitis: a randomized trial: a validation study of 2021 AASLD practice guidance for SBP. Am J Gastroenterol 2023;118(4):654–663. https://doi.org/10.14309/ajg.0000000000002126. Epub 20230103. PubMed PMID: 36594820.

[36] Patel VC, Lee S, McPhail MJW, et al. Rifaximin-α reduces gut-derived inflammation and mucin degradation in cirrhosis and encephalopathy: RIFSYS randomised controlled trial. J Hepatol 2022;76(2):332–342. https://doi.org/10.1016/j.jhep.2021.09.010. Epub 20210924. PubMed PMID: 34571050.

[37] Bajaj JS, Heuman DM, Sanyal AJ, et al. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. PloS one 2013;8(4):e60042. PubMed PMID: 32.

[38] 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 (Baltimore, Md) 2019;70(5):1690–1703. PubMed PMID: 350.

[39] Bajaj JS, Kassam Z, Fagan A, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. Hepatology (Baltimore, Md) 2017. PubMed PMID: 23.

[40] Bloom PP, Donlan J, Torres Soto M, et al. Fecal microbiota transplant improves cognition in hepatic encephalopathy and its effect varies by donor and recipient. Hepatol Commun 2022. Aug;6(8):2079–2089.

[41] Patricia P, Bloom CMB, Silber Jeffrey L, et al. Trial of a defined bacterial consortium, VE303, to treat hepatic encephalopathy. Boston, MA: AASLD, Liver Meet; 2023.

[42] Nevzorova YA, Boyer-Diaz Z, Cubero FJ, et al. Animal models for liver disease - a practical approach for translational research. J Hepatol 2020 Aug;73(2):423–440.

[43] Nguyen TL, Vieira-Silva S, Liston A, et al. How informative is the mouse for human gut microbiota research? Dis Model Mech 2015;8(1):1–16. https://doi.org/10.1242/dmm.017400. PubMed PMID: 25561744; PubMed Central PMCID: PMC4283646.

[44] Park JC, Im SH. Of men in mice: the development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. Exp Mol Med 2020;52(9):1383–1396. https://doi.org/10.1038/s12276-020-0473-2. Epub 20200910. PubMed PMID: 32908211; PubMed Central PMCID: PMC8080820.

[45] Kurtz CB, Millet YA, Puurunen MK, et al. An engineered E. coli Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. Sci Transl Med 2019;11(475). https://doi.org/10.1126/scitranslmed.aau7975. Epub 2019/01/18. PubMed PMID: 30651324.

[46] ClinicalTrials.gov. Results posting for "Safety, Tolerability and Pharmacodynamics of SYNB1020" (NCT03447730). 2021. Available from: https://clinicaltrials.gov/study/NCT03447730?term=SYNB1020&cond=cirrhosis&rank=1&tab=results.

[47] Duan Y, Llorente C, Lang S, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature 2019;575(7783):505–511. https://doi.org/10.1038/s41586-019-1742-x. Epub 2019/11/15.PubMed PMID: 31723265; PubMed Central PMCID: PMC6872939.

[48] ClinicalTrials.gov. BActeriophage to treat liver disease eliminating harmful bacteria (BATTLE) [NCT05618418] 2024 [08/19/2024]. Available from: https://clinicaltrials.gov/study/NCT05618418?cond=alcohol%20liver%20disease&term=BATTLE&intr=phage&rank=1.

[49] Administration FaD. Clinical research phase studies 2018 [3/20/2024]. Available from: https://www.fda.gov/patients/drug-development-process/step-3-clinical-research#Clinical_Research_Phase_Studies.

[50] Bajaj JS, Idilman R, Mabudian L, et al. Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. Hepatology 2018;68(1):234–247. https://doi.org/10.1002/hep.29791. Epub 20180510. PubMed PMID: 29350768.

[51] Álvares-da-Silva MR, Oliveira CP, Fagan A, et al. Interaction of microbiome, diet, and hospitalizations between Brazilian and American patients with cirrhosis. Clin Gastroenterol Hepatol 2022;20(4):930–940. https://doi.org/10.1016/j.cgh.2021.03.045. Epub 20210402. PubMed PMID: 33813071; PubMed Central PMCID: PMC8486893.

[52] Solà E, Pose E, Campion D, et al. Endpoints and design of clinical trials in patients with decompensated cirrhosis: position paper of the LiverHope Consortium. J Hepatol 2021;74(1):200–219. https://doi.org/10.1016/j.jhep.2020.08.009. Epub 20200905. PubMed PMID: 32896580.

[53] Rabiee A, Ximenes RO, Nikayin S, et al. Factors associated with health-related quality of life in patients with cirrhosis: a systematic review. Liver Int 2020. https://doi.org/10.1111/liv.14680. Epub 2020/10/01. PubMed PMID: 32998172.
[54] Tapper EB, Kanwal F, Asrani SK, et al. Patient-reported outcomes in cirrhosis: a scoping review of the literature. Hepatology 2018;67(6):2375–2383. https://doi.org/10.1002/hep.29756. Epub 20180419. PubMed PMID: 29272043.
[55] Tapper EB, Salim N, Baki J, et al. Pickle juice intervention for cirrhotic cramps reduction: the PICCLES randomized controlled trial. Am J Gastroenterol 2022;117(6):895–901. https://doi.org/10.14309/ajg.0000000000001781. Epub 20220413. PubMed PMID: 35416793.
[56] Tapper EB, Ospina E, Salim N, et al. Lactulose therapy for patients with cirrhosis, portal hypertension, and poor patient-reported outcomes: the Mi-Kristal trial. Hepatology 2023;78(4):1159–1167. https://doi.org/10.1097/hep.0000000000000408. Epub 20230418. PubMed PMID: 37066820; PubMed Central PMCID: PMC10524505.
[57] Dsouza M, Menon R, Crossette E, et al. Colonization of the live biotherapeutic product VE303 and modulation of the microbiota and metabolites in healthy volunteers. Cell Host Microbe 2022;30(4):583–598.e8. https://doi.org/10.1016/j.chom.2022.03.016. PubMed PMID: 35421353.
[58] Burnett T, Mozgunov P, Pallmann P, et al. Adding flexibility to clinical trial designs: an example-based guide to the practical use of adaptive designs. BMC Med 2020;18(1):352. https://doi.org/10.1186/s12916-020-01808-2. Epub 20201119. PubMed PMID: 33208155; PubMed Central PMCID: PMC7677786.
[59] Pallmann P, Bedding AW, Choodari-Oskooei B, et al. Adaptive designs in clinical trials: why use them, and how to run and report them. BMC Med 2018;16(1):29. https://doi.org/10.1186/s12916-018-1017-7. Epub 20180228. PubMed PMID: 29490655; PubMed Central PMCID: PMC5830330.
[60] Woodcock J, LaVange LM. Master protocols to study multiple therapies, multiple diseases, or both. N Engl J Med 2017;377(1):62–70. https://doi.org/10.1056/NEJMra1510062. PubMed PMID: 28679092.
[61] Rodriguez J, Hassani Z, Alves Costa Silva C, et al. State of the art and the future of microbiome-based biomarkers: a multidisciplinary Delphi consensus. Lancet Microbe 2024:100948. https://doi.org/10.1016/j.lanmic.2024.07.011. Epub 20240904. PubMed PMID: 39243797.
[62] de Franchis R, Bosch J, Garcia-Tsao G, et al. Baveno VII - renewing consensus in portal hypertension. J Hepatol 2022;76(4):959–974. https://doi.org/10.1016/j.jhep.2021.12.022. Epub 20211230. PubMed PMID: 35120736.
[63] Staufer K, Huber-Schönauer U, Strebinger G, et al. Ethyl glucuronide in hair detects a high rate of harmful alcohol consumption in presumed non-alcoholic fatty liver disease. J Hepatol 2022;77(4):918–930. https://doi.org/10.1016/j.jhep.2022.04.040. Epub 20220520. PubMed PMID: 35605744.
[64] Crabb DW, Im GY, Szabo G, et al. Diagnosis and treatment of alcohol-associated liver diseases: 2019 practice guidance from the American association for the study of liver diseases. Hepatology 2020;71(1):306–333. https://doi.org/10.1002/hep.30866. PubMed PMID: 31314133.
[65] Dubinkina VB, Tyakht AV, Odintsova VY, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. Microbiome 2017;5(1):141. https://doi.org/10.1186/s40168-017-0359-2. Epub 20171017. PubMed PMID: 29041989; PubMed Central PMCID: PMC5645934.
[66] Harnack L, Stevens M, Van Heel N, et al. A computer-based approach for assessing dietary supplement use in conjunction with dietary recalls. J Food Compost Anal 2008;21(Suppliment 1):S78–s82. https://doi.org/10.1016/j.jfca.2007.05.004. PubMed PMID: 19190705; PubMed Central PMCID: PMC2151738.
[67] Kim WR, Mannalithara A, Heimbach JK, et al. Meld 3.0: the model for end-stage liver disease updated for the modern era. Gastroenterology 2021;161(6):1887–1895.e4. https://doi.org/10.1053/j.gastro.2021.08.050. Epub 20210903. PubMed PMID: 34481845; PubMed Central PMCID: PMC8608337.
[68] Reiberger T, Ferlitsch A, Payer BA, et al. Non-selective betablocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. J Hepatol 2013;58(5):911–921. https://doi.org/10.1016/j.jhep.2012.12.011. Epub 20121220. PubMed PMID: 23262249.
[69] Thomson MJ, Lok ASF, Tapper EB. Appropriate and potentially inappropriate medication use in decompensated cirrhosis. Hepatology 2021;73(6):2429–2440. https://doi.org/10.1002/hep.31548. Epub 20210419. PubMed PMID: 32911564; PubMed Central PMCID: PMC7943648.
[70] Tapper EB, Henderson JB, Parikh ND, et al. Incidence of and risk factors for hepatic encephalopathy in a population-based cohort of Americans with cirrhosis. Hepatol Commun 2019;3(11):1510–1519. https://doi.org/10.1002/hep4.1425. Epub 20190906. PubMed PMID: 31701074; PubMed Central PMCID: PMC6824059.
[71] Imhann F, Bonder MJ, Vich Vila A, et al. Proton pump inhibitors affect the gut microbiome. Gut 2016;65(5):740–748. https://doi.org/10.1136/gutjnl-2015-310376. Epub 20151209. PubMed PMID: 26657899; PubMed Central PMCID: PMC4853569.
[72] Bajaj JS, Acharya C, Fagan A, et al. Proton pump inhibitor initiation and withdrawal affects gut microbiota and readmission risk in cirrhosis. Am J Gastroenterol 2018;113(8):1177–1186. https://doi.org/10.1038/s41395-018-0085-9. Epub 20180606. PubMed PMID: 29872220.
[73] Gu SL, Gong Y, Zhang J, et al. Effect of the short-term use of fluoroquinolone and β-lactam antibiotics on mouse gut microbiota. Infect Drug Resist 2020;13:4547–4558. https://doi.org/10.2147/idr.s281274. Epub 20201221. PubMed PMID: 33376361; PubMed Central PMCID: PMC7762438.
[74] Wastyk HC, Fragiadakis GK, Perelman D, et al. Gut-microbiota-targeted diets modulate human immune status. Cell 2021;184(16):4137–4153.e14. https://doi.org/10.1016/j.cell.2021.06.019. Epub 20210712. PubMed PMID: 34256014; PubMed Central PMCID: PMC9020749.
[75] Baxter NT, Schmidt AW, Venkataraman A, et al. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. mBio 2019;10(1). https://doi.org/10.1128/mBio.02566-18. Epub 20190129. PubMed PMID: 30696735; PubMed Central PMCID: PMC6355990.
[76] Venkataraman A, Sieber JR, Schmidt AW, et al. Variable responses of human microbiomes to dietary supplementation with resistant starch. Microbiome 2016;4(1):33. https://doi.org/10.1186/s40168-016-0178-x. Epub 20160629. PubMed PMID: 27357127; PubMed Central PMCID: PMC4928258.
[77] Tapper EB, Baki J, Nikirk S, et al. Medically tailored meals for the management of symptomatic ascites: the SALTYFOOD pilot randomized clinical trial. Gastroenterol Rep (Oxf) 2020;8(6):453–456. https://doi.org/10.1093/gastro/goaa059. Epub 20201112. PubMed PMID: 33442478; PubMed Central PMCID: PMC7793123.
[78] Badal BD, Fagan A, Tate V, et al. Substitution of one meat-based meal with vegetarian and vegan alternatives generates lower ammonia and alters metabolites in cirrhosis: a randomized clinical trial. Clin Transl Gastroenterol 2024;15(6):e1. https://doi.org/10.14309/ctg.0000000000000707. Epub 20240601. PubMed PMID: 38696431; PubMed Central PMCID: PMC11196077.
[79] Majdi Osman M, Zachery Stoltzner B, Kelsey O'Brien M, et al. Donor efficacy in fecal microbiota transplantation for recurrent Clostridium difficile: evidence from a 1,999-patient cohort. Infect Dis Week 2016.
[80] Danne C, Rolhion N, Sokol H. Recipient factors in faecal microbiota transplantation: one stool does not fit all. Nat Rev Gastroenterol Hepatol 2021 Jul;18(7):503–513. https://doi.org/10.1038/s41575-021-00441-5. Epub 2021/04/29. PubMed PMID: 33907321.
[81] Tap J, Lejzerowicz F, Cotillard A, et al. Global branches and local states of the human gut microbiome define associations with environmental and intrinsic factors. Nat Commun 2023;14(1):3310. https://doi.org/10.1038/s41467-023-38558-7. Epub 20230620. PubMed PMID: 37339957; PubMed Central PMCID: PMC10282066.
[82] Zhou Y, Lauschke VM. The genetic landscape of major drug metabolizing cytochrome P450 genes-an updated analysis of population-scale sequencing data. Pharmacogenomics J 2022;22(5–6):284–293. https://doi.org/10.1038/s41397-022-00288-2. Epub 20220906. PubMed PMID: 36068297; PubMed Central PMCID: PMC9674520.
[83] Kernan WN, Viscoli CM, Makuch RW, et al. Stratified randomization for clinical trials. J Clin Epidemiol 1999;52(1):19–26. https://doi.org/10.1016/s0895-4356(98)00138-3. PubMed PMID: 9973070.
[84] Administration FaD. Cfr - code of federal regulations title 21 2023. [cited 2024 4/25/2024]. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=314.3#:~:text=Active%20ingredient%20is%20any%20component,of%20man%20or%20other%20animals.
[85] Merrick B, Allen L, Masirah MZN, et al. Regulation, risk and safety of faecal microbiota transplant. Infect Prev Pract 2020;2(3):100069. https://doi.org/10.1016/j.infpip.2020.100069. Epub 20200609. PubMed PMID: 34316559; PubMed Central PMCID: PMC7280140.
[86] **DeFilipp Z**, **Bloom PP**, Torres Soto M, et al. Drug-resistant E. coli bacteremia transmitted by fecal microbiota transplant. New Engl J Med 2019. PubMed PMID: 351.
[87] Zellmer C, Sater MRA, Huntley MH, et al. Shiga toxin-producing Escherichia coli transmission via fecal microbiota transplant. Clin Infect Dis 2021

Jun 1;72(11):e876–e880. https://doi.org/10.1093/cid/ciaa1486. Epub 2020/11/08. PubMed PMID: 33159210.
[88] Administration UFaD. Safety alert regarding use of fecal microbiota for transplantation and risk of serious adverse events likely due to transmission of pathogenic organisms 2020. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse-events-likely.
[89] Smillie CS, Sauk J, Gevers D, et al. Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation. Cell Host Microbe 2018;23(2):229–240.e5. https://doi.org/10.1016/j.chom.2018.01.003. PubMed PMID: 29447696; PubMed Central PMCID: PMC8318347.
[90] Aggarwala V, Mogno I, Li Z, et al. Precise quantification of bacterial strains after fecal microbiota transplantation delineates long-term engraftment and explains outcomes. Nat Microbiol 2021;6(10):1309–1318. https://doi.org/10.1038/s41564-021-00966-0. Epub 20210927. PubMed PMID: 34580445; PubMed Central PMCID: PMC8993687.
[91] McGovern BH, Ford CB, Henn MR, et al. SER-109, an investigational microbiome drug to reduce recurrence after clostridioides difficile infection: lessons learned from a phase 2 trial. Clin Infect Dis 2021;72(12):2132–2140. https://doi.org/10.1093/cid/ciaa387. PubMed PMID: 32255488; PubMed Central PMCID: PMC8204772.
[92] Routy B, Lenehan JG, Miller Jr WH, et al. Fecal microbiota transplantation plus anti-PD-1 immunotherapy in advanced melanoma: a phase I trial. Nat Med 2023;29(8):2121–2132. https://doi.org/10.1038/s41591-023-02453-x. Epub 20230706. PubMed PMID: 37414899.
[93] Pringle PL, Soto MT, Chung RT, Hohmann E. Patients with cirrhosis require more fecal microbiota capsules to cure refractory and recurrent Clostridium difficile infections. Clin Gastroenterol Hepatol 2018. PubMed PMID: 218.
[94] Bajaj JS, Hylemon PB, Ridlon JM, et al. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. Am J physiologyGastrointestinal Liver Physiol 2012;303(6):G675–G685. PubMed PMID: 33.
[95] Fernández J, Prado V, Trebicka J, et al. Multidrug-resistant bacterial infections in patients with decompensated cirrhosis and with acute-on-chronic liver failure in Europe. J Hepatol 2019;70(3):398–411. https://doi.org/10.1016/j.jhep.2018.10.027. Epub 2018/11/06. PubMed PMID: 30391380.
[96] Rashidi A, Ebadi M, Rehman TU, et al. Long- and short-term effects of fecal microbiota transplantation on antibiotic resistance genes: results from a randomized placebo-controlled trial. Gut Microbes 2024;16(1):2327442. https://doi.org/10.1080/19490976.2024.2327442. Epub 20240313. PubMed PMID: 38478462; PubMed Central PMCID: PMC10939144.
[97] Zhang T, Gao G, Kwok LY, et al. Gut microbiome-targeted therapies for Alzheimer's disease. Gut Microbes 2023;15(2):2271613. https://doi.org/10.1080/19490976.2023.2271613. Epub 20231107. PubMed PMID: 37934614; PubMed Central PMCID: PMC10631445.
[98] Hitch TCA, Hall LJ, Walsh SK, et al. Microbiome-based interventions to modulate gut ecology and the immune system. Mucosal Immunol 2022. https://doi.org/10.1038/s41385-022-00564-1. Epub 20220930. PubMed PMID: 36180583.
[99] Franzosa EA, Sirota-Madi A, Avila-Pacheco J, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol 2019;4(2):293–305. https://doi.org/10.1038/s41564-018-0306-4. Epub 20181210. PubMed PMID: 30531976; PubMed Central PMCID: PMC6342642.
[100] Sepich-Poore GD, Zitvogel L, Straussman R, et al. The microbiome and human cancer. Science 2021;371(6536). https://doi.org/10.1126/science.abc4552. PubMed PMID: 33766858; PubMed Central PMCID: PMC8767999.
[101] Kelly CR, Yen EF, Grinspan AM, et al. Fecal microbiota transplantation is highly effective in real-world practice: initial results from the FMT national registry. Gastroenterology 2021;160(1):183–192.e3. https://doi.org/10.1053/j.gastro.2020.09.038. Epub 2020/10/05. PubMed PMID: 33011173.
[102] Cammarota G, Ianiro G, Kelly CR, et al. International consensus conference on stool banking for faecal microbiota transplantation in clinical practice. Gut 2019;68(12):2111–2121. https://doi.org/10.1136/gutjnl-2019-319548. Epub 20190928. PubMed PMID: 31563878; PubMed Central PMCID: PMC6872442.

Keywords: bacteria; probiotic; prebiotic; fecal microbiota transplant; study design; decompensation.
Received 7 May 2024; received in revised form 30 September 2024; accepted 2 October 2024; Available online 10 October 2024