The Evolution of the use of Faecal Microbiota Transplantation and Emerging Therapeutic Indications.

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1. Summary

Recent developments in high-throughput microbial genomic sequencing and other systems biology techniques have allowed novel insight into the potential contribution of the gut microbiota to health and disease. Consequently, an increasing number of disease states have been found to be characterized by distinctive changes in the composition and functionality of the gut microbiota; however, whether such changes are cause, consequence or incidental to the disease in question remains largely uncertain. Restoration of the gut microbiota to a pre-morbid state is a novel therapeutic approach of key interest, and faecal microbiota transplantation (FMT) – the transfer of pre-screened stool from healthy donors into the gastrointestinal tract of affected patients – is gaining increasing importance in both the clinical and research settings. At present, FMT is only recommended in the treatment of recurrent *Clostridioides difficile* infection, although there are a large number of ongoing trials worldwide exploring other potential therapeutic indications.

2. Introduction

Knowledge around the role of the microbiota in health and disease is rapidly expanding, with a major contributory factor being the increased availability of microbial genome sequencing.[1] The human gastrointestinal tract (GI) is inhabited by multiple different microorganisms, including bacteria, archaea, viruses and fungi. While there is no agreed definition of a healthy gut microbiota, it has been established that a healthy state is characterized by high overall microbial diversity, stability, and redundancy of key functions.[2] Perturbations of the gut microbiota (sometimes referred to as dysbiosis) have been associated with multiple diseases. As such, it is not surprising that interest in utilizing faecal microbiota transplantation (FMT) with the aim of correcting these imbalances has increased greatly.[3] FMT is the transfer of minimally manipulated pre-screened donor stool, into the gastrointestinal tract of a patient with the aim of ameliorating the dysbiotic state by increasing overall diversity and restoring the functionality of the microbiota as well.[4] Here we will review the evolution of the use of FMT and emerging therapeutic indications.

3. Established Indications for FMT*: Clostridioides difficile* Infection

3.1. Establishment of efficacy:

Clostridioides difficile infection (CDI; formerly named *Clostridium difficile*) remains a significant public health threat, as well as the most common cause of healthcare-associated infection. [4] CDI is the condition in which gut microbiota dysbiosis has been best characterized. Antibiotic use is one of the major risk factors for CDI, and antibiotic-mediated perturbation of the gut microbiota in those with CDI has been consistently described.[5]

CDI recurs in 20-30% of patients after treatment for an initial infection.[6] The paradox is that while antibiotics are known to be a major risk factor for this disease, they remain first line treatment, even for multiply recurrent CDI, which recurs despite multiple and prolonged courses of antibiotics. [7] Additionally, the failure rate of metronidazole, which previously was considered first line, has risen considerably.[8] In fact, the recently updated Infectious Disease Society of America (IDSA) guidelines removed metronidazole as a first line agent.[8]

Given the clear association between antibiotic use, gut microbiota disruption, and the development of recurrent CDI, the concept of restoration of the gut microbiota to a pre-antibiotic state arose as a potential therapeutic approach for the condition. Since the 1950s, a

growing number of case reports and case series consistently supported the principle that faecal microbiota transplant (FMT) may be a viable treatment.

Since 2013, FMT has evolved from an interesting but little-explored intervention to a mainstream therapy of global interest. This is due, in large part, to the emergence of randomized controlled clinical trials demonstrating it to be generally safe and highly-effective in the treatment of recurrent CDI; this has included a number of randomized trials that compare the efficacy of FMT to standard-of-care antibiotics (vancomycin and fidaxomicin) or placebo[9-12]. Several systematic reviews and meta-analysis have reported an overall efficacy rate between 80 and 90% of FMT in inducing cure of recurrent CDI with a single FMT. [13,14]

Two of the initial randomized trials in this field compared FMT to vancomycin in the treatment of recurrent CDI.[9,10] The first trial utilized duodenal infusions of FMT, whereas the second trial performed FMT via colonoscopy, and both trials revealed FMT to be significantly more effective then vancomycin. More recently, FMT was compared to both standard dosing of fidaxomicin and vancomycin[12]; FMT was administered both via colonoscopy and nasogastric tube, and had significantly higher efficacy then either antibiotic treatment.[12] Lastly, FMT delivery via colonoscopy was also compared to placebo (autologous stool transplant, i.e. FMT prepared from the stool of CDI patients recruited to the study).[11] Overall it was noted that FMT was superior to placebo, though among the two sites in this trial there were noticeable site differences, with one site having significant higher cure rates in the placebo group. Notably, among all these trials, no serious safety signals have been consistently identified. Across studies, the most common adverse event has been abdominal discomfort[15] . Infection transmission was an initial concern, but there has been no evidence of this in clinical practice.

Next to clinical resolution of CDI, the aim of FMT therapy is also to restore the structure and function of the gut microbiota. Recipients of FMT for rCDI have shown changes in ecological measures (including diversity and richness) and overall gut microbiota composition towards a profile similar to that of healthy donors within a day of FMT; while there is a variable degree of divergence over the course of follow-up, recipient gut microbiota profiles remain broadly-comparable to that of healthy donors for at least six months post-FMT[16] and even up to one year.[17] rCDI patients pre-FMT have consistently been shown to have lower relative abundances of the bacterial families *Lachnospiraceae, Ruminococcaceae* and *Bacteroidaceae,* and higher relative abundances of *Enterobacteriaceae, Lactobacillaceae* and *Veillonellaceae*.[18,19]

There are several important practical areas to consider when performing FMT for CDI and beyond. The recently-published '5D FMT framework for CDI' outlines each of these in detail.[4] The first consideration is the *decision*, which refers to patient selection and determining if the

patient is appropriate for FMT.[3,8,20] There are two methods of *donor* selection, patient-directed or universal donor/stool banks, which centralizes the donor identification and screening process.

The *discussion*, or informed consent, should include risks, benefits, and alternatives. Donor material can be *delivered* via nasogastric tube, colonoscopy, retention enema or capsule. Lastly the *discharge* plan should include counseling about antibiotic stewardship. [21] (**Table 1**).

3.2. Regulation of FMT:

Despite this substantial evidence regarding the safety and efficacy of FMT at treating recurrent CDI as well as its incorporation into guidelines from several societies[8,22,23], it is still not widely adopted and not FDA approved in the United States for clinical use.[24] Regulatory challenges remain one of the most complicated issues surrounding this therapy in both the US and Europe (Panel 1).

Although tight regulation is required to ensure FMT manufacturing is reaching appropriate and necessary standards, there must be balance, as excessive or burdensome regulation will prevent clinicians from providing FMT to patients who could potentially benefit (**Table 2**).

4.**Emerging Indications**

4.1 Emerging areas within CDI:

There are significant data to support the use of FMT in recurrent CDI, as reviewed above; however there are several other areas within the CDI landscape that are now being explored including severe and/or complicated CDI, as well as treatment of primary infections.

4.1.1. Severe / Complicated CDI:

Nearly 8% of hospitalized patients with CDI develop severe or fulminant disease.[31] Due to high rates of mortality in medically-refractory cases, 30% of patients with severe infection have historically undergone surgical intervention. Colectomy for this indication, even when performed in a timely fashion and by experienced surgeons, is associated with a 30-50% mortality.[32] Creation of loop-ileostomy with vancomycin lavage compared to colectomy was shown to decrease postsurgical mortality, however, it has not gained widespread popularity in the surgical community.[33] FMT emerged as a rescue therapy for these morbidly ill patients, including those not deemed to be surgical candidates. Early experience with a single FMT was prompt, but provided only temporary symptomatic improvement.[18,34] Without continuation of an anti-CDI antibiotic or repeat FMTs, these patients ultimately died or required surgery. Consequently, clinicians trialed sequential FMTs in rapid cycles, in some cases with continuation of vancomycin in-between FMTs, and described high cure rates approaching or superseding 90%.[35] A pseudomembrane-driven sequential FMT protocol, including selective use of

vancomycin until complete resolution of pseudomembranes, developed by Fischer et al. showed great promise in an uncontrolled pilot study.[36] Pseudomembranes, which are a sign of significant inflammation in the colon, are a marker of CDI severity. Independently, a similar protocol achieved 100% cure rate when patients received multiple FMTs in combination of vancomycin until complete resolution of the pseudomembranes, compared to 75% cure rate when a single FMT followed by 14 days of vancomycin therapy was given.[13] Additionally it has been reported that there is a significant decline in CDI-related colectomies after introduction of FMT for hospitalized patients.[34]

The effect of initiating an inpatient FMT program for the swift treatment of patients with severe CDI was noted in one tertiary care center in the US where CDI-related mortality decreased from 10.2% versus 4.5% in severe CDI patients and from 43.2% to 12.1% in patients with medically refractory fulminant colitis.[37] Data from retrospective and uncontrolled trials are compelling, but placebo controlled FMT trials in severe and fulminant CDI are lacking. Given that these patients are critically ill, designing placebo-controlled trials is difficult, and may even be considered unethical, given that FMT has been shown to be lifesaving. Careful consideration must be given to study design in this vulnerable patient population. Nevertheless, this therapy has shown incredible promise in this severe phenotype, and we feel any concern regarding risk in FMT would typically be outweighed by limited alternative therapeutic options and high morbidity/ mortality associated with the condition, and FMT should be offered when appropriate to do so.

4.1.2. *Primary CDI:*

The use of FMT under enforcement discretion is allowed for CDI not responding to standard-of-care antibiotics. However, the question regarding where to position FMT in the treatment paradigm remains challenging. There are limited data on the use of FMT for primary CDI. In one of the first trials comparing vancomycin to FMT, FMT was not found to be superior at achieving symptom resolution in patients with a first episode of CDI.[38] Additionally, a recently published small trial comparing FMT enemas to metronidazole did not appreciate a difference in cure rates between the two groups. Clinical cure occurred in 5 patients (56%) in the FMT group and in 5 (45%) in the metronidazole group (p=1.00).[39] It should be noted that this trial compared FMT enemas and metronidazole; enemas are associated with lower cure rates than other routes of FMT administration[40], and metronidazole has a higher failure rate compared to vancomycin.[41] Given this, this trial may not have been a fair comparison. While this is an interesting area for further exploration, the data does not currently support the use of FMT for primary CDI. Though excitement for FMT and microbiome restoration continues to grow, it is unlikely that it will be needed as first line treatment for the majority of patients. It may be a therapy reserved for those at the highest risk for recurrence.

4.2 Current status of the use of FMT in the treatment of inflammatory bowel disease:

4.2.1 CDI in Inflammatory Bowel Disease:

Inflammatory bowel disease (IBD) has been another area of significant research with regards to the potential therapeutic use of FMT. An altered microbiome has been theorized to be one of several factors contributing to the pathogenesis of IBD, but again it is unclear as to whether this is a cause or effect of the gut inflammation characterizing both Crohn's disease (CD) and ulcerative colitis (UC).[42,43] Several early case reports and case series observed the effect of FMT on IBD symptoms when utilized for the treatment of CDI.[44-46] These reports note that FMT failure rates seem to be higher in patients with IBD being treated for CDI, as well as reports of IBD flares post-FMT in this setting.[47,48] However, it difficult to assess actual flare rates from retrospective studies given that the metrics used to assess disease flares were inconsistent. The safety and efficacy of FMT in patients with both IBD and CDI is currently being assessed prospectively in an ongoing study (NCT03106844). The field continues to move forward from CDI to assess the utility of this therapy to treat IBD.

4.2.2 Ulcerative colitis:

Four randomized controlled trials (RCTs) have been published to date investigating the use of FMT in the treatment of UC, collectively including 277 patients.[49-52] The majority of participants in these studies had mild to moderate disease. These trials generally included patients with all typical disease distributions of UC, and most included patients on stable immunosuppressive therapy, although one trial excluded patients using biological therapy or methotrexate within the past two months. [49]

All four of these RCTs varied considerably in study design. Three of these studies used a lower GI administration route (enema +/- colonoscopy)[50-52], whilst one employed nasoduodenal administration.[49] Two RCTs used frozen FMT, one used fresh FMT, and one used a combination; two studies pooled stool from up to seven different donors, whilst the others administered FMT derived from individual donors. The number of FMT treatments in total varied between two and 40. Autologous FMT was used as the placebo arm in two of the studies and the others utilized brown water.

Significantly increased rates of clinical and endoscopic remission in UC patients receiving FMT compared to those receiving placebo were reported in 3 of these studies. In a recent Cochrane systematic review,[53] it was assessed that the overall remission rate at week 8 across these four studies was 37% (*n*=52/140) in patients receiving FMT, compared to 18% (*n*=24/137) in those receiving placebo (RR 2.03, 95% CI: 1.07–3.86). Rates of clinical response and endoscopic

remission also significantly improved in patients receiving FMT. The single study that used anaerobic conditions for FMT preparation reported the highest rates of steroid-free response and remission, suggesting that this may be a relevant factor to considered. One study reported a trend towards higher treatment success with one donor compared to the donors, implying that if FMT truly does have a role in the therapy of UC, donor selection may be much more important in this scenario than is the case for CDI, though the microbial characteristics necessary for an optimal donor in IBD have not been well defined. As the specific contribution of the gut microbiota to the pathogenesis of UC is increasingly understood, a future focus will be on exploring whether apparently successful donors such as this one have a gut microbiota particularly enriched in specific taxa and/or microbial functionalities that are deficient in the gut in UC.

Adverse events in participants receiving FMT for treatment of IBD were generally mild and self-limited, with common symptoms including abdominal pain, bloating, diarrhea and fever. Rates of serious adverse events (SAEs) were not significantly different between patients receiving FMT and those receiving placebo. SAEs in those receiving FMT generally reflected worsening of UC, including the need for intravenous corticosteroids and/or colectomy.

Overall, the existing evidence suggests that FMT may potentially have a role for treatment of mild to moderate UC. However, the small number of studies to date, coupled with the heterogeneity of study design, limited long-term follow-up, and relatively modest number of participants contribute to uncertainty around the efficacy and safety of FMT in patients with UC. Of note there are case reports/case series exploring the role of FMT in severe UC, but it difficult to assess the significance of these studies. There is currently no consensus regarding where FMT will fit in the UC treatment paradigm, especially when a number of novel immunomodulatory medications are also becoming of increasing clinical importance. In addition, the majority of the RCTs performed in this space have been underpowered. As such, recent guidelines and consensus documents recommend that, at this time, FMT should only be performed for UC in the context of a clinical trial.[23,54]

4.2.3 Crohn's disease:

At the time of writing, there have been no randomized controlled studies evaluating the use of FMT in the treatment of Crohn's disease, although a number of relatively small cohort studies, including both adults and children, have been published. A recent meta-analysis of eleven studies, including four case reports and seven cohort studies, reported an overall 50.5% (*n*=42/83) rate of clinical remission, and few serious adverse events attributable to the FMT.[55] However, the marked heterogeneity of the distribution and activity of disease and FMT administration protocols of the included studies limited the interpretability of findings. A

double-blind randomized controlled trial evaluating the efficacy of FMT in adults with Crohn's disease is ongoing (NCT03078803).

5. Future Directions and Areas of Uncertainty :

There are several other indications that are being explored as potential targets for FMT therapy with variable results. Here we will review a few promising indications with available trial data. Other emerging areas of particular interest are summarized in **Table 3A**. At the time of writing, there are over 200 registered trials investigating the use of FMT to treat various disorders on clinicaltrials.gov (Table 3B).

5.1 Hepatological indications:

Hepatic encephalopathy (HE), a common complication of end stage liver disease, is another condition that has been characterized by an altered gut microbiota, and where treatments that modulate the gut microbiota (including lactulose and rifaximin) already have an established role in therapy for the condition.[56] The stool microbiota of HE patients has a reduced relative abundance of beneficial short-chain fatty acid (SCFA) producing families such as *Lachnospiraceae* and *Ruminococcaceae*, and enrichment of potentially-pathogenic *Enterobacteriaceae*. This microbial profile has been linked to cognitive impairment and systemic inflammation seen in HE. [57] Given this basis, FMT has been another therapy of interest for potentially reversing the disturbed 'gut-brain axis' that characterizes the condition. A single RCT compared patients on standard of care treatment with lactulose and rifaximin (*n*=10), to those who received five days of antibiotics followed by a single FMT enema in addition to standard of care (*n*=10). [58] Patients who received FMT were noted to have significantly fewer HE episodes as well as improved cognitive testing compared to controls by day 150. The primary outcome of this trial was safety and notably most of the safety events seen in the standard of care arm were hepatic encephalopathy events. The authors noted the benefits seen with FMT were maintained for up to 12 months. Furthermore, no SAEs clearly related to FMT were noted.

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease, often linked to IBD, that currently has no approved medical therapies. PSC is characterized by a risk of a number of malignancies (particularly cholangiocarcinoma and colorectal carcinoma), as well as a risk chronic liver disease and its sequelae; liver transplantation is the only definitive treatment for the condition. PSC patients were shown to have gut dysbiosis that is distinctive from that of patients with IBD alone. [59] The promise of FMT in UC raised the possibility of efficacy in patients with PSC and IBD. A single open label trial has been conducted of patients with a confirmed diagnosis of PSC and baseline alkaline phosphatase >1.5 times the upper limit of normal in the setting of IBD. Ten patients underwent a single FMT via colonoscopy and were followed for 6

months. [60] In this cohort, 3 patients (30%) experienced a 50% decrease in alkaline phosphatase by 6 months. This trial is a promising step into understanding the microbial contribution to the pathogenesis of this disease, a disease that currently has limited therapeutic options, with larger trials planned.

5.2 Irritable Bowel Syndrome/Functional Bowel Disorders:

Irritable bowel syndrome (IBS) is a constellation of disorders that include alterations in the form or frequency of stool and associated abdominal pain. Overall, five RCTs have been performed assessing FMT for the treatment of IBS.[61-65] One of the double-blinded RCT of patients with IBS-diarrhea type and mixed (diarrhea and constipation) received FMT via colonoscopy (*n*=60) or placebo (*n*=30). A higher proportion of patients experienced relief from their IBS symptoms at 3 months in the arm that received donor FMT compared to autologous FMT as placebo (65% (*n*=36/55) vs 43% (*n*=12/28) respectively, *p*=0.049).

However, in another double-blind RCT including patients with all forms of moderate to severe IBS, patients were treated with FMT capsules or equivalent placebo capsules. IBS symptom scores were significantly lower and quality of life scores were better in patients treated with placebo (*n*=26) compared to those treated with FMT capsules (*n*=25) (*p*=0.012). No SAEs clearly related to FMT occurred in any study.

The results from all five trials provide mixed results for the utility of FMT in this patient population, further studies on more defined subsets of IBS patients may be necessary to understand if FMT is efficacious in this disorder. These trials are difficult to compare given the heterogeneous design, follow up and outcomes defined in these trials. It is unclear if results were related to method of administration (ie: colonoscopy and need for bowel prep) as opposed to the FMT itself.

5.3 Metabolic syndrome:

Metabolic syndrome and obesity affect millions of people globally.[66] Alterations in the gut microbiota have been linked to both obesity and insulin resistance.[67] Two randomized trials (*n*=56 total) demonstrated significant improvements in peripheral, but not hepatic, insulin sensitivity in male patients with metabolic syndrome at six weeks post-FMT using stool derived from lean donors. However, this response was not maintained past six weeks. No changes were observed in patients who received autologous FMT as placebo.[68,69] Specifically, the first trial (2012) was a pilot randomized controlled trial (n=18) in male patients with metabolic syndrome (BMI >30 kg/m^2, waist circumference >102cm, fasting plasma glucose level >5.6mmol/l). Participants received FMT from either a lean donor (BMI <23 kg/m^2) or autologous stool

transfer. Among participants who received a lean FMT, insulin sensitivity increased (median rate of glucose disappearance changed from 26.2 to 45.3 μmol/kg/min; p<0.05). More recently (2017) this trial was replicated with a randomized controlled trial (n=38) in male patients with metabolic syndrome ((BMI >30 kg/m^2, waist circumference >102cm, fasting plasma glucose level >5.6mmol/l, triglycerides >=1.7 mmol/l, HDL <1.03 mmol/l, blood pressure >=130/85mmHg) receiving FMT either a lean donor (BMI <25 kg/m^2) or autologous stool transfer. At 6 weeks, participants who received lean FMT, insulin sensitivity increased (median rate of glucose disappearance changed from 25.8 to 28.8 μmol/kg/min; p<0.05); however, the result did not persist to week 18. [68,69]

More recently, a double blind RCT (*n*=20), in which patients with metabolic syndrome received either FMT from a lean vegan donor (*n*=10) or autologous FMT (*n*=10) showed no changes in lean vegan FMT recipients in either the production capacity of the atherogenic metabolite trimethylamine N-oxide (TMAO) or proxies of vascular inflammation.[70]

5.4 'Next generation' FMT for recurrent CDI:

An initial proof-of-concept trial ("RePOOPulate") revealed that a stool substitute (comprising purified bacterial cultures of 33 commensal isolates from health donor stool) was successful at treating CDI.[71] This led to increased commercial interest and industry-funded trials to assess FMT-like products for the treatment of recurrent CDI. These trials, however, have had variable success, which was initially surprising given the overwhelming success of FMT in both open-label and randomized controlled trials.

There are other full spectrum preparations currently under investigation (NCT03110133) with promising early studies[75]; however, to date, we do not have an industry-developed product that has emerged from the standard FDA drug pathway (Panel 2).

The mechanisms underlying the efficacy of FMT in treating recurrent CDI remain incompletely understood, and this has become an area of considerable recent interest. Given that whole stool FMT or even defined consortia may not be appropriate or preferred for certain patients (such as the severely immunocompromised), preparations without live organisms have been investigated.[76] In recurrent CDI, the FMT-mediated restoration of a pre-morbid gut bile acid milieu, mediated by microbial bile-metabolizing enzymes (particularly bile salt hydrolases), appears to be a key mechanism of efficacy. [19] There is also recent evidence that FMT restores SCFAs within the colon, with the SCFA valerate directly preventing vegetative growth of *C. difficile.*[77] However, there are likely to be a number of contributory mechanisms of action[80,81]

6. Future directions and conclusion

The emergence of randomized trials demonstrating the marked efficacy of FMT (compared to antimicrobial therapy and placebo) in the treatment of recurrent CDI has led gastroenterologists, microbiologists/ infectious diseases clinicians and other relevant stakeholders to rapidly establish FMT services globally. However, FMT clearly presents very unique and complex challenges to clinicians and regulators alike, including its poorly-defined mechanism of action, the complexities associated with donor selection and the use of a human product, and the lack of long-term follow-up data (**Table 2**).

Many of these unknowns regarding FMT, while recognized as concerning, generally have been deemed acceptable as the potential benefit in the treatment of patients with multiply recurrent CDI (who are often frail, elderly patients with very limited alternative therapeutic options) often outweighs the risk. However, such concerns are substantially amplified in the era where FMT is expanding to the non-CDI setting, and especially as FMT is being used as an investigational tool in younger patients with chronic diseases. There are currently many ongoing trials assessing the safety and efficacy of FMT in various conditions, but many of these trials are small pilot studies without control arms, as investigators are being asked to do pilot safety studies for each new disease being assessed. Furthermore, it is frequently unclear what the appropriate control arms in these trials should be, and whether autologous FMTs are comparable to a true placebo.

Whilst conventional pharmaceuticals undergo extensive testing for quality control in the consistency of their production before entering clinical trials, FMT is clearly unique in the potential variability in microbial characteristics between donors, and even in different stool samples from the same donor over time. Pharmaceuticals have well-established pharmacokinetic and pharmacodynamic profiles, but this does not exist for FMT; furthermore, in trials using FMT for non-CDI indications, there are many outstanding questions surrounding optimal mode and frequency of administration, and the answer may depend on the specific diagnosis being treated. There have been early attempts to pick stool donors rationally, via selection based on gut microbiota function known to be lacking in the disease state of interest, but donor selection is often mandated by stool availability limited to ability to personalize donor selection.

Progress toward answering these unknowns are underway (**Table 2**), and questions regarding long-term safety remains a high priority. A recently published study with 3.8 years of follow up found FMT to be safe and durable. Additional follow-up data is currently limited (although no significant safety signals have consistently been observed), but this is currently being assessed by an NIH-funded patient registry, which will prospectively collect efficacy and safety data in as many as 4000 patients over up to 10 years (NCT03325855). Whilst donor stool has been historically chosen based principally on practicality and availability, there has been the tentative

emergence of donor stool selection (at least in the CDI setting) based on microbiota characteristics, which has potential translatability to non-CDI FMT trials. Perhaps most fundamentally, there is the need for further mechanistic studies to better define the specific contribution that altered gut microbiota composition has for the pathogenesis of the huge range of non-CDI conditions in which it has been observed.

Our understanding of the composition and function of the gut microbiota and the potential for its manipulation to cure disease is an area of rapid growth and great promise. FMT has allowed for deeper investigation into the potential role that the gut microbiota may be playing in several chronic and difficult-to-treat disorders with the hopes that unlocking a key understanding of the pathogenesis of these diseases will allow for the development of more targeted therapies. While defined consortia and other approaches to 'next generation FMT' are being investigated, at present there is no alternative to whole-stool FMT. In ten years, it seems unlikely we will still be performing whole FMT, but rather that it will be replaced with the development of more personalized or even synthetic microbial therapies. However, medical research has not been able to create a synthetic blood product, and FMT may turn out to be similar; synthetic reproduction of this complex ecosystem may not be possible. In either case, we hope that the FDA and other regulatory bodies do not prevent providers from offering this incredibly-effective therapy to patients with recurrent CDI, as this remains the most effective therapy available currently with an excellent safety profile. Further research will result in better understanding around the mechanisms of action of FMT, improved FMT preparations and modes of administration, donor selection for different disease states, appropriate length of follow-up, and the complex issues surrounding regulation. Within this space, there is much to learn and novel gut microbial therapeutics are likely to rapidly evolve over the next decade.

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Table 1

Table 1: Summary of the '5D framework' on optimal use of FMT as treatment for recurrent CDI: As adapted from Allegretti et al GIE 2018[4]

Category:	**Details:**
Decision	Decision regarding patient eligibility. Treating clinician should review indications as well as all available CDI test results. Currently eligible patients include: • 3rd or further confirmed occurrence of CDI • 2nd episode if both required hospitalization • **Note**: Not currently recommended for 1st episode CDI.
Donor	Donor material is either patient directed, meaning a single donor is provided by the patient is screened or obtained from a universal stool bank. This is a process in which healthy volunteers are screened regularly and stool is banked for future use. Most have adopted a universal banking model either at their institution or by utilizing a stool bank. This allows stool to be screened more thoroughly and in advance to not delay care • Broadly, donors should be screened using robust health questionnaires, blood and stool testing in keeping with best practices. • Frozen material yields equivalent cure rates as fresh material[82,83].
Discussion	Review the risks, benefits and alternatives to FMT. • No serious adverse events definitely attributed to FMT have been identified • Common mild adverse events include: diarrhea, abdominal cramps. Nausea, fever, bloating, gas, and constipation
Delivery	Delivery method will differ based on clinical context. There are advantages and disadvantages to each route of delivery and should be considered on a case by case basis. Modalities include: • Upper GI delivery: upper endoscopy, naso-enteric tubes, or capsules • Lower GI delivery: colonoscopy, flexible sigmoidoscopy or enema.
Discharge	After FMT patients should be counseled about proper cleaning of high touch surface areas as well as be taught about antibiotic stewardship. • Patients should be followed after FMT to assess for adverse events • Patients should be assessed through week 8 post FMT to assess for recurrence of CDI. Cure is defined as absence of diarrhea at week 8 post FMT or if diarrhea is present negative testing using a two-step testing algorithm that include and enzyme immunoassay test for toxin.

Table 2: Current uncertain areas and future directions for FMT:

Topic:	Description of the problem:	Potential future directions:
Mechanisms of action	• FMT has significant practical drawbacks, including unpalatability, the potential need for invasive administration, a theoretical risk of transmission of infection, etc; understanding mechanisms of action may facilitate novel targeted therapeutics. • Safety of a therapy involving live microorganisms remains unclear in certain patient groups, e.g. severely immunosuppressed. • Currently relatively-limited insight into mechanisms of action of FMT, even in the context of recurrent CDI. • Potential limitations in translatability of microbiota studies between rodents and humans due to marked differences in structure and function of gut microbiota.	• Capsulized FMT has helped overcome concerns of invasive administration, but not other drawbacks. Transendoscopic enteral tubing administration is also being explored.[84] • Further studies defining changes in gut microbiota profile, metabolic function and host immunological profile in human participants in clinical trials between pre- and post-FMT, and using this as a basis to mechanistic studies of the contribution of the gut microbiota to the condition. • Use of advanced systems biology techniques (e.g. shotgun sequencing of microbial genomes, metabonomics, proteomics, etc) to track individual strains between donor and recipient, and direct linkage to impact upon microbiota-host interactions. •
FMT preparation and administration	• Only relative limited understanding about optimal means of FMT administration, role for anaerobic prep, safe length of time and temperature for storing FMT in freezer etc. • Clear evidence from laboratory studies of impact of these variables and others upon the structure and functionality of the stool microbiota.[85,86] • Preparation used may influence interpretability of clinical trials of FMT for non-CDI indications, e.g. does a negative primary outcome reflect purely a suboptimal means of FMT preparation and administration, or true absence of efficacy?	• Further translational research to explore specific influence of such variables upon microbial and metabolic profile of FMT. • Investigation of the impact of FMT preparation upon clinical outcomes in randomized trials, e.g. use of anaerobic preparation.[52]
Donor selection	• Donor screening is laborious and expensive. Even relatively minor perturbations in health (e.g. short course of oral antibiotics) can result in at least temporary exclusion of donors from donor pool. • Small but appreciable failure rate of FMT even for recurrent CDI, with lack of clarity of whether this relates to donor or recipient factors. • Donors selected after relatively crude risk factor/ laboratory screening; limited consensus definition of a 'healthy microbiota' which may biologically	• Development of stool banks/ 'hub and spoke' FMT services (central centre preparing FMT providing to an entire region) streamlines the process of maintaining a donor pool. • Further mechanistic investigation of the potential contribution of the gut microbiota to various disease states may improve donor-recipient matching.

	guide matching of donor and recipient.	
Length of follow-up	• Relatively novelty in clinical use of FMT means that only limited long-term follow-up clinical data. • Theoretical concern about FMT transmitting gut microbiota trait from donor to recipient associated with potential increased future risk of disease, e.g. type 2 diabetes mellitus. • FMT trials increasingly including conditions prominent in younger patients (e.g. IBD), and/or children.	• Establishment of FMT registry data (e.g. AGA FMT national registry, [NCT03325855]) for early recognition of potential concerns. • Most FMT regulatory bodies/ guidelines recommend long-term storage of donor serum/ stool for potential 'look back' exercises in case of future concerns.
Regulation	• Regulation established on country-by-country basis without uniform agreement on to what extent FMT should be regulated comparably to conventional medicinal products. • FMT services and/ or stool banks in many regions increasingly being required to obtain specialist licenses as quality assurance before allowed to supply; costs and complexity in obtaining licenses may limit expansion of FMT services.	• Ongoing dialogue between clinicians experienced in FMT and relevant regulatory bodies to clarify regulatory pathways. • May require differential regulation of FMT for CDI with FMT in clinical trials.

Table 3A: Summary of major non-CDI disease states in which FMT has been assessed as potential therapy:

Indication:	Key data:
Intestinal carriage of multi-drug resistant organisms[87-89]	• Successful FMT for rCDI was shown to be associated with a significant reduction in antibiotic-resistance genes within the stool microbiota of recipient. • A number of subsequent case reports and case series support FMT having a potential role in intestinal decolonisation of a range of multi-drug resistant organisms, even in immunosuppressed patients. • However, in a randomised trial, rates of gut colonisation with ESBL- and/or carbapenemase-producing *Enterobacteriaceae* were not reduced in immunocompetent adults receiving five days of oral antibiotics (colistin and neomycin) followed by FMT compared to controls receiving no intervention (decolonisation in 41% (*n*=9/22) in FMT arm vs 29% (*n*=5/17) in control arm).
Haematopoietic stem cell transplant (HSCT) recipients[90-93]	• *Antibiotic use:* o Allo-HSCT recipients often require antibiotics, and low gut microbial diversity predicts increased mortality in these patients. o In an RCT, 'auto-FMT' (i.e. FMT of own stool banked before treatment) in 14 patients restored gut microbiota composition and diversity comparable to that of patient's pre-allo-HSCT profile. • *Graft vs host disease (GvHD):* o Case reports and small case series have demonstrated at least transient improvements in selected patients with acute and chronic intestinal GvHD (including steroid-refractory and steroid-dependent patients), in terms of progression-free survival, GI symptoms and/or corticosteroid use. o No serious adverse events clearly linked to FMT reported.
Autistic spectrum disorders (ASD)[94]	• One open label trial: o 18 children with ASD and moderate to severe gastrointestinal problems, aged 7-16 years, treated with two weeks of vancomycin, a bowel purge, and then 7-8 weeks of FMT. Either oral or rectal FMT as the initial administration at high dose, followed by low dose daily oral FMT for the remainder of the study. o GI symptom scores and ASD behavior scores had both significantly improved by the end of FMT administration, and these improved scores were still maintained at week 18 after commencement of the study (i.e. at least weeks after completing FMT administration).
Inflammatory bowel disease[49-52]	• See main text (**Section 4.2**).
Irritable bowel syndrome (IBD)[61,62]	• See main text (**Section 5.2**).
Hepatic encephalopathy (HE)[58]	• See main text (**Section 5.1**).
Primary sclerosing cholangitis[60]	• See main text (**Section 5.1**).
Metabolic syndrome[68-70]	• See main text (**Section 5.3**).

Table 3B: Selected ongoing randomised trials of FMT as experimental therapy for non-CDI conditions:

Indication	Trial number design and primary centre	Trial details
Bipolar disorder	NCT03279224, randomized, double-blind, placebo-controlled trial; Toronto, Canada	• 60 adults with bipolar disorder, randomized in 1:1 ratio to colonoscopic healthy donor FMT (using donor with no personal or family history of significant psychiatric history) or autologous FMT. • Primary outcome of change in the Montgomery-Asberg depression rating scale between baseline and that at week 24 post-FMT.
Cirrhosis	NCT02862249, randomised placebo-controlled trial; London, UK	• 32 cirrhotic adults with MELD score of 10-16, randomised in ratio of 3:1 to nasojejunal healthy donor FMT or placebo (normal saline/ glycerol). • Primary outcome measures of safety and feasibility.
Malnutrition	NCT03087097, randomised, double-blind, placebo-controlled trial; Cape Town, South Africa	• 20 children (18-60 months of age) with severe acute malnutrition not responsive to standard therapy, randomised in ratio of 1:1 to enema administration of healthy donor FMT or placebo. • Primary outcome of safety, secondary outcomes including changes in leptin levels.
Parkinson's disease	NCT03808389, randomised, double-blind, placebo-controlled trial; Ghent, Belgium	• 40 adults with Parkinson's disease randomised in ratio of 1:1 to nasojejunal healthy donor FMT or placebo (autologous FMT). • Primary outcome of clinical symptoms as scored on the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPRS) at up to 12 months.
Psoriatic arthritis	NCT03058900, randomised, double-blind, placebo-controlled trial; Odense, Denmark	• 80 adults with peripheral psoriatic arthritis (≥3 swollen joints despite ≥3 months of methotrexate), randomised in ratio of 1:1 to upper GI-administered health donor FMT or placebo (normal saline). All patients will continue weekly methotrexate throughout. • Primary outcome of treatment failure within six months of FMT, e.g. need for escalation to biologic therapy.

Panel 1

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Panel 2

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Search Strategy

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