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Review Article

Fecal Microbiota Transplantation for Treatment of Acute Graft-*versus*-Host Disease

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ABSTRACT

The growing understanding of the bidirectional relationship between the gastrointestinal (GI) microbiome and the immune system has opened up new avenues for treatment of graft-*versus*-host disease (GVHD). Fecal microbiota transplantation (FMT) is the transfer of stool from a donor to a recipient who harbors a perturbed GI microbiome resulting in disease. We review the rationale for performing FMT for the treatment of acute GVHD, and summarize data on the safety and efficacy of the procedure among allogeneic hematopoietic stem cell transplantation (HSCT) recipients. Overall, FMT is a promising strategy in treating and preventing HSCT-related complications. However, caution should be exerted as HSCT recipients are highly immunosuppressed and unanticipated infectious adverse events may appear with the increasing application of FMT.

1. BACKGROUND

1.1. Acute Graft-*versus*-Host Disease

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative procedure for a variety of hematological malignancies. The transplantation process involves administration of a conditioning regimen, which includes chemotherapeutic agents with or without radiation, followed by infusion of hematopoietic progenitor cells from healthy donors [1]. Following conditioning, blood counts go down, and approximately two to three weeks after transplantation the stem cells engraft, and the slow process of immune reconstitution begins. Despite advances in supportive care and transplantation technology, graft-*versus*-host disease (GVHD) remains a major cause of transplantation-related mortality and morbidity, affecting up to 40–60% of allo-HSCT patients, and accounting for 15–20% of deaths [2]. GVHD is traditionally divided into an acute and chronic disease, and in this review, we will focus on the acute form which involves the skin, gut, or liver. Acute GVHD typically appears in the first 100 days posttransplantation, but can also develop later, especially in patients receiving reduced-intensity conditioning or direct lymphocyte infusions.

Acute GVHD pathophysiology has been reviewed elsewhere [3–5]. Briefly, alloreactive T-cells transplanted from a nonidentical donor recognize the transplant recipient as foreign, thereby initiating an immune reaction damaging the recipient's tissues. GVHD can be considered a three-step process in which the innate and adaptive immune systems interact: (1) tissue damage to the recipient by the radiation/chemotherapy pretransplant conditioning regimen, (2) donor T-cell activation and clonal expansion, and (3) activation of cytotoxic T-cells and natural killer cells, inflicting local tissue damage. There is a growing body of evidence implicating the gut microbiota in the development and propagation of GVHD [6,7].

1.2. The Microbiome in HSCT

The human body is colonized by a multitude of microorganisms. Microbiota refers to the entirety of microorganisms (bacteria, archaea, viruses, fungi, and other eukaryotes) within a specific habitat. Microbiome is defined as the biotic (microorganisms and their genomes) and abiotic (environmental) factors present within a particular habitat. Work led by the groups of Marcel van den Brink and Eric Pamer has revealed intriguing associations between alterations of the gut microbiota and outcomes of allogeneic HSCT, including infections, mortality, and relapse [8–12]. Since the gut microbiota and its metabolites are essential for development and maturation of the host immune system and modulation of the immune response [13–16], it is unlikely that changes in the microbiome during and following HSCT lack functional implications. As the insult to the gut during conditioning is instrumental for the development of GVHD, and the gut is a target organ in acute GVHD, there is a strong rationale to postulate that the gut

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microbiome plays a major role in GVHD through its interaction with the immune system. Furthermore, intestinal homeostasis and mucosal integrity are dependent on the microbial community occupying the gut [15,17].

The first studies linking the microbiota to GVHD were performed almost half a century ago. Germ-free mice and antibiotic-treated mice were less likely to develop GVHD and experienced longer survival [18–21]. An attempt to reproduce these findings in humans through gut decontamination, laminar-airflow isolation rooms, and skin cleansing led to mixed results [22–28]. More recently, the advent of high-throughput molecular methods to study the microbiome has provided new insights. Several groups have reported that dysbiosis and a reduction in stool bacterial diversity following allogeneic HSCT are associated with increased risk of acute GVHD and GVHD-related mortality [8,29,30]. Interestingly, depending on the cohort, stool samples of patients who later developed acute GVHD were enriched for specific bacterial taxa; intestinal domination by *enterococci* and a reduction in *Blautia* spp., as well as other members of the *Clostridia* class, were apparent before and during GVHD development [8,30]. The protective role of commensal anaerobes was further supported by an increased risk for GVHD in patients treated with broad-spectrum antibiotics with coverage of anaerobic bacteria [31,32]. Other bacteria implicated in GVHD include members of the *Bacteroides* genus (*B. thetaiotaomicron, B. ovatus*, and *B. caccae*) which were negatively correlated with subsequent severe acute GVHD in stool samples collected at the time of neutrophil engraftment. *Rothia mucilaginosa, Solobacterium moorei*, and *Veillonella parvula* were also positively correlated, while several *Lachnospiraceae* (including *B. luti*) and a *Butyricicoccus* species were negatively correlated [29]. The gut microbiome is a complex biosystem and, therefore, assuming that specific species drive GVHD risk might be simplistic. Golob *et al.* suggested that the gradient between protective and detrimental species is more predictive of acute GVHD [29].

Whether and how the microbiota contributes to the development of GVHD development and propagation remains an open question. Since the immune system and the microbiota have a robust bidirectional relationship [33–37], it is reasonable to postulate that injury to the gastrointestinal (GI) mucosa secondary to conditioning and antibiotics is essential for GVHD evolution. Increased permeability of the intestine leads to bacterial translocation from the gut lumen, which in turn stimulate the innate immune system via toll-like receptors, priming alloreactive T-cells that drive GVHD [6,36,38]. Metabolites of commensal bacteria may also modulate the activation of the immune response. For instance, certain *Clostridia* species, which might be reduced during transplantation owing to the use of antibiotics with aerobic coverage, exert an anti-inflammatory effect by production of the short chain fatty acid butyrate, thereby relieving or preventing intestinal acute GVHD [39]. Several papers have reviewed the link between GVHD, microbiota, and its metabolites [6,7,17].

Severe acute GVHD often involves the GI system [40]. Patients refractory to treatment with glucocorticosteroids, considered the first-line therapy, have a dismal prognosis with survival rates as low as 5% [40,41]. Currently, there is no standard for second-line therapy [42]. Therefore, novel insights into the prevention and treatment of GI acute GVHD are urgently needed. Knowledge on the role of the microbiome in GVHD pathophysiology have opened up a new avenue of therapeutic targets [7]. Antibiotics, prebiotics (indigestible compounds that are fermented by commensal bacteria to produce protective metabolites such as short-chain fatty acids), probiotics (multiple or selected strains of microorganisms that confer a benefit), postbiotics (bacterial metabolites), and dietary interventions [43,44] are all areas of intense research. In this review, we will focus on fecal microbiota transplantation (FMT), an intervention that has pre-, pro-, and postbiotic elements, and has emerged as a promising live microbial therapy for GI acute GVHD.

2. FECAL MICROBIOTA TRANSPLANTATION

2.1. Principle and Current Use

FMT is the transfer of stool from a donor to a recipient that harbors a dysbiotic or perturbed GI microbiome resulting in disease. By introducing a "healthy" microbiota, FMT aims to restore eubiosis and homeostasis in the recipient. Modern use of FMT began in 1958: Eiseman and colleagues described four patients with Staphylococcal pseudomembranous enterocolitis who improved after fecal enemas [45]. FMT was abandoned for many years until its resurgence for treatment of *Clostridioides* (formerly Clostridium) *difficile* infection (CDI), first reported by Schwan *et al.* in 1983 [46]. Since then, multiple studies have demonstrated that FMT is a highly effective treatment for recurrent CDI [47–51]. Mechanisms underlying FMT efficacy have been reviewed by Khoruts and Sadowsky, and include restoration of the colonic microbial community and inhibition of *C. difficile* by competition for nutrients, direct suppression by antimicrobial peptides, bile-acid-mediated inhibition of spore germination and vegetative growth, and activation of immune-mediated colonization resistance [52].

Fecal donation may be from related or nonrelated donors or precollected autologous stools. Donors undergo screening for infectious disease as well as other comorbid conditions. Centralized stool banks are advocated to ensure safety and permit the use of fecal donation across multiple centers [53]. FMT may be administered via either colonoscopy, nasogastric/duodenal tubes, capsules, or enema; all routes of administration are effective in recurrent CDI [47–51]. Enemas and capsules are less invasive, but the spread through the distal GI tract and the inoculum size in the latter are reduced. Furthermore, there is concern that they may be less effective in repleting certain classes, including Bacteroidia [54], which may have GVHD protective properties. Nasogastric/duodenal tube or colonoscopy allows for administration of large amounts of fecal matter, but are invasive [55]. The process of preparing the fecal inoculum is similar across delivery methods and includes mixing and grinding of the stool with normal saline, removing particulate matter, and storage in an appropriate vehicle for delivery.

FMT is an accepted therapy for recurrent CDI, and has been endorsed by national and professional societies and regulatory authorities across the globe [56]. Thousands of stool transplantation have been performed worldwide with an excellent safety profile. Typically, FMT adverse events are mild and include bloating, flatulence, nausea, and abdominal discomfort. Severe adverse events, including infections, are rare and are mainly related to the risks of nasogastric tube insertion, sedation, and endoscopy [57]. There is a theoretical concern of transmission of infectious

pathogens not identified in the screening process. Furthermore, data regarding potential long-term effects, including transmission of noninfectious donor disease (e.g., metabolic syndrome) and immune dysregulation are lacking.

2.2. FMT for Treatment of *C. Difficile* in Immunocompromised Patients

Immunocompromised patients represent a unique population where a greater concern for infectious complications exists. Kelly *et al.* studied outcomes of 80 immunocompromised patients treated with FMT owing to CDI [58]. Causes for immunosuppression included HIV/AIDS, solid organ transplants, oncologic conditions, immunosuppressive therapy for inflammatory bowel disease, and other medical conditions/medications. Severe complications following FMT were rare and related to aspiration during sedation and mucosal tear during colonoscopy. No infectious adverse events related to FMT were observed. Other studies have reported similar findings with low rates of FMT-related infections [59–65]. Overall, FMT appears safe in immunocompromised patients. However, data are limited and experience with HSCT recipients, which represent the far extreme of the immunosuppressed population, is lacking.

FMT in the HSCT setting was initially reported for treatment of recurrent CDI (Table 1). Webb *et al.* treated seven allogeneic HSCT recipients with FMT, administered via a nasojejunal tube. CDI resolved in all patients, and the procedure was well tolerated with no serious adverse events or any infectious complications. Interestingly, one patient with acute GI GVHD was able to taper systemic steroids following the FMT. Moss *et al.*, described eight patients who were previously treated with autologous or allogeneic HSCT who developed recurrent CDI. All patients were treated with FMT in capsules and had complete resolution of the infection [64]. Additional case reports and case series have described favorable outcomes in HSCT patients treated with FMT for recurrent CDI [66–69].

2.3. Fecal Microbiota Transplantation for Microbiota Restoration and Eradication of Antibiotic-Resistant Bacteria in HSCT Recipients

FMT has also been explored as a mean of restoring microbiota injury following HSCT (Table 2). The rationale is that FMT may reduce dysbiosis following HSCT, which is associated with poor transplantation outcomes [9]. Furthermore, FMT may diminish hospital-acquired infections and carriage of antibiotic-resistant bacterial strains, which are prevalent in immunocompromised patients [64,70,71]. In a single-center pilot study, 13 patients undergoing allogeneic HSCT received FMT from an unrelated donor via capsules. FMT was performed no later than four weeks after neutrophil engraftment [72]. The procedure was well tolerated without severe adverse events. FMT increased intestinal bacterial diversity. Following FMT 1, 2, and 6 patients developed *C. difficile* colitis, grades 3–4 GI acute GVHD, and moderate-severe chronic GVHD, respectively. Taur *et al.* performed a randomized controlled open-label trial of autologous FMT via enema *versus* no intervention in allo-HSCT recipients; the results of the first 25 of 59 patients have been reported [73]. Stool for FMT was collected before HSCT hospitalization; patients were only considered eligible for the trial if a "healthy" intestinal bacterial profile was observed. FMT was performed following neutrophil engraftment. FMT was safe and boosted microbial diversity, restoring bacterial populations lost during HSCT and reversing the disruptive effects of the broad-spectrum antibiotics. Two studies directly used FMT to eradicate multidrug-resistant bacteria. Bilinski *et al.* performed FMT via a nasoduodenal tube in 20 patients with various blood disorders, some of which were also HSCT recipients who were colonized with antibiotic-resistant bacteria [74]. Again, FMT was well tolerated without infectious complications; antibiotic-resistant bacteria were successfully eradicated in 75% of the patients. Similar findings were reported by Battipaglia *et al.*, who performed FMT by enema or

Table 1 | FMT studies in HSCT recipients for the treatment of CDI.

Study	Indication/ Population	Number of Patients	Administration Route	Study Type	Donor Relation	Total Number of FMTs	Adverse Events	Response
Neeman *et al.*[68]	Severe fulminant CDI/ allo-HSCT	1	Naso-jejunal	Case report	Husband	1	No serious AEs	1/1 resolution of CDI
de Castro *et al.*[69]	Recurrent CDI/ allo-HSCT	1	Push enteroscopy	Case report	Unrelated	1	No serious AEs	1/1 no recurrence of CDI
Mittal *et al.*[67]	Recurrent CDI/ auto-HSCT	1	Enema	Case report	Unrelated	2	No serious AEs	1/1 no recurrence of CDI
Webb *et al.*[62]	Recurrent CDI allo-HSCT	7	Naso-jejunal tube/-colonoscopy	Retrospective, case series	Unrelated	8	No serious AEs	6/7 no recurrence of CDI
Moss *et al.*[64]	Recurrent CDI allo/auto HSCT	8	Oral capsules	Retrospective, case series	Unrelated	8	No serious AEs	8/8 no recurrence of CDI
Bluestone *et al.*[66]	Recurrent CDI	3	Gastric tube/-colonoscopy	Retrospective, case series	Relative/ unrelated	3	No serious AEs	1/3 no recurrence of CDI

Abbreviation: FMT: Fecal microbiota transplantation, HSCT, Hematopoietic stem cell transplantation, CDI: Clostridioides difficile infection, Allo: Allogenic, Auto: Autologous, AE: Adverse event.

nasogastric tube in 10 patients before (n = 4) or after (n = 6) allogeneic HSCT [75]. Overall, the bulk of data indicate that FMT is safe in HSCT recipients and its use may extend beyond the treatment of recurrent CDI.

2.4. Fecal Microbiota Transplantation for Treatment of GI Acute GVHD

Given the link between GI acute GVHD and the microbiota, FMT has been investigated as a potential therapeutic intervention (Tables 3 and 4). In their pioneering study, Kakihana *et al.* performed FMT for treatment of steroid-resistant (n = 3) or steroid-dependent (n = 1) acute GI GVHD [76]. The FMT construct was fresh (i.e., not frozen and then thawed) and donors were relatives. At the time of FMT, patients had at least grade II acute GVHD, were on a minimal dose of 1 mg/kg methylprednisolone and received tacrolimus and beclomethasone. Patients received 1–2 courses of FMT via a nasoduodenal tube. Three patients achieved complete normalization of GI symptoms, and one patient experienced transient relief in diarrhea and was defined as having partial response. Response to FMT correlated with increased peripheral circulating regulatory T-cells and intestinal bacterial diversity, with the appearance of beneficial

Table 2 | FMT studies in HSCT recipients for restoring gut microbiota and eradication of antibiotic-resistant bacteria.

Study	Indication	Number of Patients	Administration Route	Study Type	Donor Relation	Total Number of FMTs	Adverse Events	Response/ Endpoint
Bilinski *et al.*[74]	Multidrug-resistant bacteria decolonization	20 (n = 8 allo-HSCT recipient; n = 12 other hematologic conditions)	Nasoduodenal tube	Prospective	Unrelated	25	No serious AEs	15/20 decolonization of multidrug-resistant bacteria
DeFilipp *et al.*[72]	Gut microbiota reconstitution following allo-HSCT	13	Oral capsules	Prospective	Unrelated	13	1 abdominal pain	Improved microbiome diversity
Taur *et al.*[73]	Gut microbiota reconstitution following allo-HSCT	25 (n = 14 received auto FMT; n = 11 no intervention)	Enema	Randomized controlled trial	Autologous FMT	25	No serious AEs	Restored gut microbiota to pre allo-HSCT state
Battipaglia *et al.*[75]	Multidrug-resistant bacteria decolonization	10 (n = 6 after allo-HSCT; n = 4 before allo-HSCT)	Enema/ nasogastric tube	Retrospective	Unrelated/ relative	13 (n = 9 after allo-HSCT)	No serious AEs	7/10 decolonization of multidrug-resistant bacteria

Abbreviation: FMT: Fecal microbiota transplantation, HSCT: Hematopoietic stem cell transplantation, Allo: Allogenic, GVHD: Fraft-*versus*-host disease, AE: Adverse event.

Table 3 | FMT studies in HSCT recipients for the treatment of GVHD.

Study	Indication/ Population	Number of Patients	Administration Route	Study Type	Donor Relation	Total Number of FMTs	Adverse Events	Response
Kakihana *et al.* [76]	Steroid resistant/ dependent gut GVHD	4	Nasogastric tube	Prospective	Spouse /relative	7	1- lower GI bleeding, hypoxemia (probably not related)	n = 3, CR; n = 1, PR
Spindelboeck *et al.* [78]	Steroid resistant grade IV gut GVHD	3	Colonoscopy	Retrospective, case series	Unrelated/ sibling	9	No serious AEs	n = 2, CR; n = 1, PR
Qi *et al.* (NCT03148743) [79]	Steroid-resistant GvHD	8	Nasoduodenal tube	Prospective	Unrelated	12	No serious AEs	n = 5, CR; n = 1, PR
Shouval *et al.* (NCT 03214289) [81]	Steroid-resistant/ dependent GVHD	7	Oral capsules	Prospective	Unrelated	15	2-Bacteremia (deemed unrelated)	n = 2, CR
van Lier *et al.* [80]	Steroid-resistant/ dependent GVHD	15	Nasoduodenal tube	Prospective	Unrelated	15	No serious AEs	n = 11, CR

Abbreviation: FMT: Fecal microbiota transplantation, HSCT: Hematopoietic stem cell transplantation, GVHD: Graft-*versus*-host disease, CR: Complete response, PR: Partial response, AE: Adverse event.

Table 4 | List of ongoing trials for treatment and prevention of acute GVHD with fecal microbiota transplantation.

	Title	Aim	Route of Administration	Design, *N*, Location
NCT03819803	Fecal microbiota transplantation in a GVHD after ASCT	FMT for treatment of steroid-dependent/resistant GI acute GVHD	Colonoscopy	Single arm, *N* = 15, Austria
NCT03812705	Fecal microbiota transplantation for steroid-resistant/dependent acute GI GVHD (FEMITGIGVHD)	FMT for treatment of steroid-dependent/resistant GI acute GVHD	Colonoscopy or gastroscopy	Single arm, Phase 2, *N* = 30, China
NCT03549676	Fecal microbiota transplantation for treatment of refractory graft-*versus*-host disease: a pilot study	FMT for treatment of steroid dependent/resistant GI Acute GVHD (pediatric population)	Naso-jejunal tube	Single arm, Phase 1, *N* = 15, China
NCGT03492502	Autologous fecal microbiota transplantation for patients with acute graft-*versus*-host disease	Autologous FMT for treatment of steroid-dependent/resistant GI acute GVHD	n/a	Single arm, *N* = 70, Israel
NCT03214289	Fecal microbiota transplantation for steroid-resistant and steroid-dependent gut acute graft-*versus*-host disease	FMT for treatment of steroid-dependent/resistant GI acute GVHD	Oral capsules	Single arm, Phase I, *N* = 4, Israel
NCT03148743	Fecal microbiota transplantation in gut a GVHD	FMT for treatment of GI acute GVHD	Naso-duodenal tube	Single arm, Phase I, *N* = 20, China
NCT03359980	Treatment of steroid refractory gastrointestinal Acute GVHD after allogeneic HSCT with fecal microbiota transfer (HERACLES)	Fecal microbiota transfer for treatment of steroid-dependent/resistant GI acute GVHD	Enema	Single arm, Phase II, *N* = 32, Europe
NCT03720392	Fecal microbiota transplantation (FMT) in recipients after allogeneic hematopoietic cell transplantation (HCT)	FMT for prevention of complications and microbiota restoration in allo-HSCT recipients	Oral capsules	Two arms, phase II, randomized, *N* = 48, United States
NCT03678493	A study of FMT in patients with AML allo HSCT in recipients	FMT for prevention of complications and microbiota restoration in allo-HSCT recipients and AML patients	Oral capsules	Four arms, Phase II, randomized, double-blind, placebo-controlled, N = 120, United States

Abbreviation: FMT: Fecal microbiota transplantation, GI: Gastrointestinal, GVHD: Graft-*versus*-host disease, Allo-HSCT: Allogeneic hematopoietic stem cell transplantation, AML: Acute myeloid leukemia.

species such as *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, and *Faecalibacterium* spp., which are characteristic of a "healthy" microbiota. One patient developed mild hypoxemia, delirium, and lower GI bleeding a few days after the FMT. The same patient experienced transplant-associated thrombotic microangiopathy three days after the second FMT. The authors attributed these events to refractory GVHD rather than the FMT. Three of the four patients in this pilot study eventually died, with disease relapse as the cause of death in two. A theoretical concern that FMT mitigates the graft-*versus*-tumor effect could be raised. However, these were patients at high risk of relapse without a control arm to provide a valid comparison. Furthermore, prolonged immunosuppression may also increase relapse [77]. Spindelboeck *et al.* treated three patients with grade IV GI acute GVHD with repeated FMTs via colonoscopy [78]. Two achieved clinical response after 1–6 courses of FMT. Two patients had a complete response, and one a transient reduction in diarrheal symptoms. No severe adverse events were observed. In the most recent study by Qi *et al.*, eight patients with stage IV GI acute GVHD received 1–2 courses of FMT from an unrelated donor via nasoduodenal tube [79]. All patients achieved clinical symptomatic remission after the first FMT. Three patients experienced relapse of GI symptoms. No severe adverse events were noted. Following FMT, intestinal bacterial diversity increased in the recipients, and so did abundance of beneficial bacteria such as *Bacteroides*. Two abstracts have been published reporting on FMT outcomes in steroid-resistant acute GI GVHD. van Lier *et al.*, performed FMTs from unrelated donors via nasoduodenal infusion [80]. Eleven of 15 patients showed complete response. GI GVHD eventually recurred in five of the responding patients. 16S ribosomal RNA sequencing of fecal samples from the first 13 patients revealed overall low alpha diversity (i.e., microbiota diversity within the samples) in patients pre-FMT. One week after FMT, the fecal microbial composition of patients in complete remission resembled that of the donor. We have also reported our experience with repeated courses of FMT from unrelated donors, administered via frozen capsules, in steroid-resistant (n = 6) and steroid-dependent (n = 1) patients [81]. Two exhibited complete clinical response. Capsules were well tolerated. Notably, two patients developed bacteremia a few days following the FMT. In the first case, using metagenomic sequencing allowing strain-level identification, the *Enterococcus Faecium* recovered

from the patient's blood was already present in the host gut in a sample collected prior to the FMT. In the second case of *Pseudomonas aeruginosa* bacteremia, 16S rRNA sequencing did not detect the bacteria in the FMT inoculum. With a high degree of certainty, these infectious events were not directly related to the FMT. Possibly, the FMT might have altered intestinal permeability, thereby increasing the risk of infections, as suggested by Quera *et al.*, who also reported a case of bacteremia following FMT in a patient with Crohn's disease [82]. Overall, FMT seems to be a promising approach for GI acute GVHD. Nevertheless, caution should be exerted, since this is a highly immunosuppressed population. In addition, to allow for persistent microbiota engraftment, antibiotics should be withheld. This may prove as a difficult task in steroid-resistant GVHD patients, who are often on antibacterial therapy for prevention or treatment of infections.

3. FUTURE DIRECTIONS

The role of FMT in HSCT and, in particular, in gut acute GVHD continues to evolve. So far, studies have focused on treatment of steroid-resistant GVHD, which carry a dismal prognosis. Indeed, regardless of response to treatment, survival was short in six out of the seven patients reported by Kakihana *et al.* and Spindelboeck [76,78]. Therefore, FMT should preferably be introduced for prevention or early intervention in the course of acute GVHD. In addition, randomized clinical trials are critical. Examples of drugs showing preliminary efficacy in the treatment of steroid-resistant GVHD in early studies but not in phase III trials are not infrequent [42]. The major points which will have to be addressed in future studies on FMT in HSCT recipients include FMT donor selection, route of administration, timing, dosing, and frequency of the procedure, as more than one course may be required [78]. Also needed are better definitions of outcomes and safety measures, as patients with GVHD resistant to therapy are prone to develop severe complications, including infections, transplantation-associated microangiopathy, and relapse. Given the landscape of competing events, determining which events are related to FMT and whether the patient responded to therapy may be difficult. Surrogate biomarkers such as inflammatory biomarkers and cytokines may prove useful in this regard. Our understanding of the link between the microbiome and GVHD is growing. Strategies for customizing the FMT to specific recipients, with matching according to the microbial or metabolic profile of the inoculum may improve the procedure's efficacy and safety. Furthermore, microbiota-specific approaches may come forward, with administration of carefully designed bacterial agents.

CONFLICT OF INTERESTS

Authors have not relevant competing interests to disclose.

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CONTRIBUTORS

R.S. collected the literature. R.S and M.G. wrote the first version of the manuscript. A.N. and I.Y. critically reviewed the manuscript, and made a substantial contribution to the interpretation of the data and the final text

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REFERENCES

[1] Copelan, EA. Hematopoietic stem-cell transplantation. N Engl J Med 2006;354(17);1813–26.
[2] Jagasia, M, *et al.* Risk factors for acute GVHD and survival after hematopoietic cell transplantation. Blood 2012;119(1);296–307.
[3] Zeiser, R, Blazar, BR. Acute graft-versus-host disease—biologic process, prevention, and therapy. N Engl J Med 2017;377(22); 2167–79.
[4] Holtan, SG, Pasquini, M, Weisdorf, DJ. Acute graft-versus-host disease: a bench-to-bedside update. Blood 2014;124(3);363–73.
[5] Ferrara, JL, *et al.* Graft-versus-host disease. Lancet 2009; 373(9674);1550–61.
[6] Staffas, A, Burgos da Silva, M, van den Brink, MR. The intestinal microbiota in allogeneic hematopoietic cell transplant and graft-versus-host disease. Blood 2017;129(8);927–33.
[7] Shono, Y, van den Brink, MRM. Gut microbiota injury in allogeneic haematopoietic stem cell transplantation. Nat Rev Cancer 2018;18(5);283–95.
[8] Jenq, RR, *et al.* Intestinal blautia is associated with reduced death from graft-versus-host disease. Biol Blood Marrow Transplant 2015;21(8);1373–83.
[9] Taur, Y, *et al.* The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood 2014;124(7);1174–82.
[10] Taur, Y, *et al.* Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis 2012;55(7);905–14.
[11] Ubeda, C, *et al.* Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. J Clin Invest 2010;120(12);4332–41.
[12] Peled, JU, *et al.* Intestinal microbiota and relapse after hematopoietic-cell transplantation. J Clin Oncol 2017;35(15); 1650–9.
[13] Thaiss, CA, *et al.* The microbiome and innate immunity. Nature 2016;535(7610);65–74.
[14] Gomez de Aguero, M, et al. The maternal microbiota drives early postnatal innate immune development. Science 2016;351(6279); 1296–302.
[15] Zelante, T, *et al.* Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 2013;39(2);372–85.
[16] Cox, LM, *et al.* Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell 2014;158(4);705–21.
[17] Kohler, N, Zeiser, R. Intestinal microbiota influence immune tolerance post allogeneic hematopoietic cell transplantation and intestinal GVHD. Front Immunol 2018;9;3179.
[18] Connell, MS, Wilson, R. The treatment of x-irradiated germfree cfw and c3h mice with isologous and homologous bone marrow. Life Sci (1962) 1965;4;721–9.

[19] Jones, JM, Wilson, R, Bealmear, PM. Mortality and gross pathology of secondary disease in germfree mouse radiation chimeras. Radiat Res 1971;45(3);577–88.
[20] van Bekkum, DW, Knaan, S. Role of bacterial microflora in development of intestinal lesions from graft-versus-host reaction. J Natl Cancer Inst 1977;58(3);787–90.
[21] Heit, H, *et al.* Allogeneic bone marrow transplantation in conventional mice: I. Effect of antibiotic therapy on long term survival of allogeneic chimeras. Blut 1977;35(2);143–53.
[22] Buckner, CD, *et al.* Protective environment for marrow transplant recipients: a prospective study. Ann Intern Med 1978;89(6); 893–901.
[23] Navari, RM, *et al.* Prophylaxis of infection in patients with aplastic anemia receiving allogeneic marrow transplants. Am J Med 1984;76(4);564–72.
[24] Petersen, FB, *et al.* Laminar air flow isolation and decontamination: a prospective randomized study of the effects of prophylactic systemic antibiotics in bone marrow transplant patients. Infection 1986;14(3);115–21.
[25] Petersen, FB, *et al.* Prevention of nosocomial infections in marrow transplant patients: a prospective randomized comparison of systemic antibiotics versus granulocyte transfusions. Infect Control 1986;7(12);586–92.
[26] Beelen, DW, *et al.* Evidence that sustained growth suppression of intestinal anaerobic bacteria reduces the risk of acute graft-versus-host disease after sibling marrow transplantation. Blood 1992;80(10);2668–76.
[27] Beelen, DW, *et al.* Influence of intestinal bacterial decontamination using metronidazole and ciprofloxacin or ciprofloxacin alone on the development of acute graft-versus-host disease after marrow transplantation in patients with hematologic malignancies: final results and long-term follow-up of an open-label prospective randomized trial. Blood 1999;93(10);3267–75.
[28] Vossen, JM, *et al.* Complete suppression of the gut microbiome prevents acute graft-versus-host disease following allogeneic bone marrow transplantation. Plos One 2014;9(9);e105706.
[29] Golob, JL, *et al.* Stool microbiota at neutrophil recovery is predictive for severe acute graft vs host disease after hematopoietic cell transplantation. Clin Infect Dis 2017;65(12); 1984–91.
[30] Holler, E, *et al.* Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. Biol Blood Marrow Transplant 2014;20(5);640–5.
[31] Shono, Y, *et al.* Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl Med 2016;8(339);339ra71.
[32] Nishi, K, *et al.* Impact of the use and type of antibiotics on acute graft-versus-host disease. Biol Blood Marrow Transplant 2018;24(11);2178–83.
[33] Belkaid, Y, Hand, TW. Role of the microbiota in immunity and inflammation. Cell 2014;157(1);121–41.
[34] Atarashi, K, *et al.* Treg induction by a rationally selected mixture of clostridia strains from the human microbiota. Nature 2013;500(7461);232–6.
[35] Smith, PM, *et al.* The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 2013; 341(6145);569–73.
[36] Vinolo, MA, *et al.* Regulation of inflammation by short chain fatty acids. Nutrients 2011;3(10);858–76.
[37] Noor, F, *et al.* The gut microbiota and hematopoietic stem cell transplantation: challenges and potentials. J Innate Immun 2018;1–11.
[38] Zhao, Y, *et al.* TLR4 inactivation protects from graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Cell Mol Immunol 2013;10(2);165.
[39] Mathewson, ND, *et al.* Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. Nat Immunol 2016;17(5);505–13.
[40] Castilla-Llorente, C, *et al.* Prognostic factors and outcomes of severe gastrointestinal GVHD after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant 2014;49(7);966–71.
[41] Westin, JR, *et al.* Steroid-refractory acute GVHD: predictors and outcomes. Adv Hematol 2011;2011;601953.
[42] Martin, PJ, *et al.* First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant 2012;18(8);1150–63.
[43] Peled, JU, *et al.* Role of gut flora after bone marrow transplantation. Nat Microbiol 2016;1;16036.
[44] Andermann, TM, *et al.* The microbiome and hematopoietic cell transplantation: past, present, and future. Biol Blood Marrow Transplant 2018;24(7);1322–40.
[45] Eiseman, B, *et al.* Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery 1958;44(5);854–9.
[46] Schwan, A, *et al.* Relapsing clostridium difficile enterocolitis cured by rectal infusion of homologous faeces. Lancet 1983; 2(8354);845.
[47] van Nood, E, *et al.* Duodenal infusion of donor feces for recurrent clostridium difficile. N Engl J Med 2013;368(5);407–15.
[48] Youngster, I, *et al.* Oral, capsulized, frozen fecal microbiota transplantation for relapsing clostridium difficile infection. JAMA 2014;312(17);1772–8.
[49] Cammarota, G, *et al.* Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent costridium difficile infection. Aliment Pharmacol Ther 2015;41(9);835–43.
[50] Kelly, CR, *et al.* Effect of fecal microbiota transplantation on recurrence in multiply recurrent clostridium difficile infection: a randomized trial. Ann Intern Med 2016;165(9);609–16.
[51] Kao, D, *et al.* Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent clostridium difficile infection: a randomized clinical trial. JAMA 2017;318(20); 1985–93.
[52] Khoruts, A, Sadowsky, MJ. Understanding the mechanisms of faecal microbiota transplantation. Nat Rev Gastroenterol Hepatol 2016;13(9);508–16.
[53] Terveer, EM, *et al.* Faecal microbiota transplantation in clinical practice. Gut 2018;67(1);196.
[54] Jiang, ZD, *et al.* Safety and preliminary efficacy of orally administered lyophilized fecal microbiota product compared with frozen product given by enema for recurrent clostridium difficile infection: a randomized clinical trial. PLOS ONE 2018;13(11);e0205064.
[55] Vindigni, SM, Surawicz, CM. Fecal microbiota transplantation. Gastroenterol Clin North Am 2017;46(1);171–85.
[56] McDonald, LC, *et al.* Clinical practice guidelines for clostridium difficile infection in adults and children: 2017 update by the

Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis 2018;66(7);987–94.
[57] Wang, S, *et al.* Systematic review: adverse events of fecal microbiota transplantation. Plos One 2016;11(8);e0161174.
[58] Kelly, CR, *et al.* Fecal microbiota transplant for treatment of clostridium difficile infection in immunocompromised patients. Am J Gastroenterol 2014;109(7);1065–71.
[59] Shogbesan, O, *et al.* A systematic review of the efficacy and safety of fecal microbiota transplant for clostridium difficile infection in immunocompromised patients. Can J Gastroenterol Hepatol 2018;2018;1394379.
[60] Elopre, L, Rodriguez, M. Fecal microbiota therapy for recurrent clostridium difficile infection in HIV-infected persons. Ann Intern Med 2013;158(10);779–80.
[61] Friedman-Moraco, RJ, *et al.* Fecal microbiota transplantation for refractory clostridium difficile colitis in solid organ transplant recipients. Am J Transplant 2014;14(2);477–80.
[62] Webb, BJ, *et al.* Fecal microbiota transplantation for recurrent clostridium difficile infection in hematopoietic stem cell transplant recipients. Transpl Infect Dis 2016;18(4);628–33.
[63] Alrabaa, S, *et al.* Fecal microbiota transplantation outcomes in immunocompetent and immunocompromised patients: a single-center experience. Transpl Infect Dis 2017;19(4), e12726.
[64] Moss, EL, *et al.* Long-term taxonomic and functional divergence from donor bacterial strains following fecal microbiota transplantation in immunocompromised patients. Plos One 2017;12(8);e0182585.
[65] Lin, SC, Alonso, CD, Moss, AC. Fecal microbiota transplantation for recurrent clostridium difficile infection in patients with solid organ transplants: an institutional experience and review of the literature. Transpl Infect Dis 2018;20(6);e12967.
[66] Bluestone, H, Kronman, MP, Suskind, DL. Fecal microbiota transplantation for recurrent clostridium difficile infections in pediatric hematopoietic stem cell transplant recipients. J Pediatric Infect Dis Soc 2018;7(1);e6–e8.
[67] Mittal, C, *et al.* Fecal microbiota transplant for recurrent clostridium difficile infection after peripheral autologous stem cell transplant for diffuse large B-cell lymphoma. Bone Marrow Transplant 2015;50(7);1010.
[68] Neemann, K, *et al.* Fecal microbiota transplantation for fulminant clostridium difficile infection in an allogeneic stem cell transplant patient. Transpl Infect Dis 2012;14(6);E161–E5.
[69] de Castro Jr, CG, *et al.* Fecal microbiota transplant after hematopoietic SCT: report of a successful case. Bone Marrow Transplant 2015;50(1);145.
[70] DeFilipp, Z, *et al.* Fecal microbiota transplantation: restoring the injured microbiome after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2019;25(1);e17–e22.
[71] Baker, TM, Satlin, MJ. The growing threat of multidrug-resistant gram-negative infections in patients with hematologic malignancies. Leuk Lymphoma 2016;57(10);2245–58.
[72] DeFilipp, Z, *et al.* Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity. Blood Adv 2018;2(7);745–53.
[73] Taur, Y, *et al.* Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. Sci Transl Med 2018;10(460);eaap9489.
[74] Bilinski, J, *et al.* Fecal microbiota transplantation in patients with blood disorders inhibits gut colonization with antibiotic-resistant bacteria: results of a prospective, single-center study. Clin Infect Dis 2017;65(3);364–370.
[75] Battipaglia, G, *et al.* Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematological malignancies carrying multidrug-resistance bacteria. Haematologica 2019;2018;198549.
[76] Kakihana, K, *et al.* Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. Blood 2016;128(16);2083–8.
[77] Inamoto, Y, *et al.* Influence of immunosuppressive treatment on risk of recurrent malignancy after allogeneic hematopoietic cell transplantation. Blood 2011;118(2);456–63.
[78] Spindelboeck, W, *et al.* Repeated fecal microbiota transplantations attenuate diarrhea and lead to sustained changes in the fecal microbiota in acute, refractory gastrointestinal graft-versus-host-disease. Haematologica 2017;102(5);e210–e13.
[79] Qi, X, *et al.* Treating steroid refractory intestinal acute graft-vs.-host disease with fecal microbiota transplantation: a pilot study. Front Immunol 2018;9;2195.
[80] van Lier,YF, et al. Fecal microbiota transplantation can cure steroid-refractory intestinal graft-versus-host disease. Biol Blood Marrow Transplant 2019;25(3);S241.
[81] Shouval, R, et al. Repeated courses of orally administered fecal microbiota transplantation for the treatment of steroid resistant and steroid dependent intestinal acute graft Vs. host disease: a pilot study (NCT 03214289). Blood 2018;132(Suppl 1);2121.
[82] Quera, R, *et al.* Bacteremia as an adverse event of fecal microbiota transplantation in a patient with Crohn's disease and recurrent clostridium difficile infection. J Crohns Colitis 2014;8(3);252–3.