REVIEW ARTICLE

The clinical role of the gut microbiome and fecal microbiota transplantation in allogeneic stem cell transplantation

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ABSTRACT

Outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) have improved in the recent decade; however, infections and graft-*versus*-host disease remain two leading complications significantly contributing to early transplant-related mortality. In past years, the human intestinal microbial composition (microbiota) has been found to be associated with various disease states, including cancer, response to cancer immunotherapy and to modulate the gut innate and adaptive immune response. In the setting of allo-HSCT, the intestinal microbiota diversity and composition appear to have an impact on infection risk, mortality and overall survival. Microbial metabolites have been shown to contribute to the health and integrity of intestinal epithelial cells during inflammation, thus mitigating graft-*versus*-host disease in animal models. While the cause-and-effect relationship between the intestinal microbiota and transplant-associated complications has not yet been fully elucidated, the above findings have already resulted in the implementation of various interventions aiming to restore the intestinal microbiota diversity and composition. Among others, these interventions include the administration of fecal microbiota transplantation. The present review, based on published data, is intended to define the role of the latter approach in the setting of allo-HSCT.

Introduction

The past decades have witnessed important advances in the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT),[1] mainly attributed to the reduction in non-relapse mortality.[2] Yet, the need for further improvement is compelling. Acute graft-*versus*-host disease (aGvHD) and infections are two of the main causes of early transplant-related mortality (TRM), jointly accounting for 36% and 43% of deaths by day 100 in matched related and matched unrelated transplants, respectively.[1]

One of the emerging and extensively explored allo-HSCT-associated issues is the change in the gut microbial flora, as well as its effect on the pathogenesis of transplant-related complications and association with transplant outcomes.

The human body hosts a hundred trillion microbial organisms; the majority of them are bacteria, predominantly colonizing the gut, with the lower intestine being most densely colonized (10^{11}-10^{12} organisms/g of intestinal content).[3] The composition of bacteria in the gut is referred to as the intestinal microbiota and their collective genome is termed the "intestinal microbiome".[3] The two main phyla constituting more than 90% of the gut microbiota are the *Firmicutes* and *Bacteroidetes* and among less dominant phyla are *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia*.[4] This composition is relatively flexible and can rapidly change in response to different environmental factors, adjusting the metabolic and immunologic performance accordingly.[5] Intestinal microbiota has been recently found to have a significant impact on both health and disease states. It appears to be crucial for the maturation and education of the immune system and has a role in intestinal cell proliferation, intestine vascularization and endocrine functions. Moreover, it produces energy, synthesizes vitamins, metabolizes bile acids and even inactivates drugs.[6-13] The microbiome has been reported to be associated with a variety of disorders such as

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obesity, type 2 diabetes, inflammatory bowel disease, rheumatoid arthritis and multiple sclerosis.[14-17] This association is also suggested to be true for cancer[18] and response to cancer immunotherapy.[19] The gut microbiota has a close and reciprocal relationship with the host immune system. Intestinal epithelial cells, goblet and paneth cells produce the luminal protective mucosal layer and antimicrobial peptides, allowing the transcellular transport of immunoglobulin A (IgA) antibodies. These functions regulate luminal microbial colonization.[20]

Homeostasis of the immune response in the gut mucosa is maintained by the balance between pro-inflammatory cells, which include T-helper 1 (Th1) cells producing interferon γ (IFNγ), Th17 cells producing IL-17A and IL-22, diverse innate lymphoid cells with cytokine effector features resembling those of Th2 and Th17 cells, the anti-inflammatory Foxp3+ regulatory T-cells (Tregs) and IgA-secreting B-cells. This homeostasis can be modulated by the gut microbiota.[21-23] In pre-clinical studies, intestinal microbiota has been shown to regulate the expression of pro-inflammatory cytokines, human leukocyte antigen (HLA) type I and type II molecules and increase T-cell proliferation.[18] Effects of the microbiota on cytokine expression and immune cell subsets are not limited to the gut, and are extended to regional mesenteric and systemic lymph nodes.[24] Furthermore, while some bacterial strains can induce pro-inflammatory intestinal Th17 cells,[25] others induce anti-inflammatory Tregs[26,27] and can thus ameliorate inflammatory colitis.[28] Moreover, human host gut microbiota has been shown to correlate with expression pattern of the cytokines secreted from peripheral blood mononuclear cells isolated from the host.[29] Microbial metabolites such as the short chain fatty acid (SCFA) butyrate or indole derivatives produced by tryptophan metabolism act to maintain the intestinal epithelial cell health, mucosal barrier, and to promote anti-inflammatory responses.[30,31]

Currently available molecular techniques allowing rapid and wide genomic sequencing enable extensive exploration of the microbiome. The most commonly used method is the 16S ribosomal RNA sequencing by PCR. Bioinformatics analysis tools assign the sequences to microbial taxon at different taxonomic levels. Other methods include shotgun next-generation metagenomics sequencing enabling massive and deeper genomic sequencing and allowing better identification of taxonomic species and potential functional pathways of the organisms, metatranscriptomics using high throughput RNA sequencing to profile gene expression, metaproteomics capable to provide large-scale characterization of the entire proteins in the environmental sample and metabolomics, identifying and quantifying all metabolites in the tested samples.[32,33] The two main microbiome features that have been widely characterized in health and disease are its diversity and the abundance of specific bacteria or bacterial subgroups.[34]

The revelation of significant relationship between the microbiome, the immune system and disease has led to interventional studies aiming to normalize the microbiome composition and diversity thus ameliorating disease conditions. One of such interventions is the use of fecal microbiota transplantation (FMT), the term referring to the transfer of the fecal microbial content from a healthy individual into the intestine of a diseased individual. FMT, the standard of care for refractory or recurrent *Clostridium difficile* infection (CDI), proved to be highly effective in this condition. At the same time, mixed results were demonstrated in the studies evaluating the use of FMT for the management of inflammatory bowel disease, irritable bowel syndrome and hepatic encephalopathy. To date, FMT application for indications other than CDI has been limited to the experimental setting only.[35,36]

The setting of allo-HSCT imposes a significant disruption on the gut microbiome homeostasis through a variety of mechanisms (all part of the transplantation procedure), such as the use of broad-spectrum antibiotics, dietary changes (restriction), gut epithelial damage by conditioning regimens and introduction of a donor immune system.

Data from clinical studies support the association of alterations in the gut microbiome profile, mainly loss of diversity and change in composition during allo-HSCT, with patient outcomes such as aGvHD, GvHD-related mortality, non-relapse mortality (NRM) and overall survival (OS).[37-40] Moreover, the gut microbial composition is reported to have an impact on infection risk, including CDI and blood stream infections (BSI), in this clinical setting.[38,41] Findings of these associations have led to a preponderance of research in this field,[42] and although the cause-and-effect relationship between the microbiome and transplant complications has not been unequivocally established, many ongoing clinical trials are implementing various interventions aiming to maintain microbiome diversity, thus potentially preventing transplant-related complications and treating aGvHD. These interventions include the use of probiotics,[43] prebiotics,[44] change in antibiotic prophylaxis[45] and administration of FMT.[46] This review appraises the currently available evidence on the association of gut microbiota and allo-HSCT and analyzes a potential role of FMT in allo-HSCT, by presenting two illustrative clinical cases, where effects on the gut microbiota composition could be employed either as a prophylactic or therapeutic measure.

Case 1

A 54-year old male, with mutated FLT3-ITD acute myeloid leukemia (AML) in complete remission (CR) after induction and re-induction chemotherapies, during which he acquired gut colonization with carbapenem-resistant *Klebsiella pneumoniae*. He underwent an allo-HSCT from a mismatched 9/10 unrelated female donor with myeloablative conditioning (busulfan, fludarabine) and received levofloxacin for infection prophylaxis. During the transplantation period, he had a BSI event with extended spectrum β lactamase *Escherichia coli* (*E. coli*) treated with meropenem for 10 days, followed by a CDI event treated with oral vancomycin. His neutrophils engrafted on day +15 and on day +33 he developed diarrhea and was diagnosed with grade 3 acute lower gastrointestinal (GI) GvHD that was steroid refractory.

This case raises a number of important questions related to the role of gut flora in allo-HSCT.

Is the microbiome already disrupted prior to allogeneic hematopoietic stem cell transplantation conditioning?

There is ample evidence suggesting that the pre-transplant patient microbiome is already disrupted. The insult to the microbiome starts with preceding chemotherapy and

antibiotic exposure. Galloway-Pena *et al.*[47] analyzed 487 stool samples from 30 AML patients and found that their pre-induction microbiome diversity was not significantly different from that of healthy volunteers participating in the Human Microbiome Project (HMP). However, following neutrophil recovery, patient microbiome composition changed, with a significant decrease in diversity. Importantly, this reduction in diversity was associated with an increased risk of infections. The use of carbapenem antibiotics for more than 3 days during induction elevated the risk for a subsequent loss of diversity.[47] Moreover, exposure to anti-anaerobic antibiotics, like piperacillin-tazobactam, ticarcillin, meropenem, clindamycin and metronidazole, within the 3 months preceding allo-HSCT was associated with a significant decrease in pre-transplant microbiome diversity.[38] With more courses of intensive chemotherapy, such as re-induction or salvage, the microbiome disruption was shown to enhance, leading to ecosystem instability and outgrowth of pathogenic bacteria like *Enterococcus*.[48] This disruption in patient microbiome continued up to the time of allo-HSCT, as shown in the largest to date inter-center effort, where 8,767 sequential stool samples were collected from 1,362 patients prior to and throughout the transplantation period and analyzed using 16S ribosomal RNA sequencing. The pre-transplant microbiome of patients obtained on days -30 to -6 (n=606), was compared to that of healthy volunteers (n=246), demonstrating a significant reduction in diversity in patient microbiome.[37] Additionally, evidence from another recently published study showed that the pre-transplant microbiome and the one derived from healthy controls differed in composition, displaying decreased abundance of beneficial bacteria of genera *Bifidobacterium* and butyrate producing genera such as *Faecalibacterium* and *Lachnospiraceae* in the former case.[49] To conclude, pre-transplant microbiome disruption is clearly evident.

What is the microbiome status during the transplantation period and at time of recovery?

Data from several studies demonstrate that during the transplantation course, the microbiome diversity significantly decreases and its composition changes.[37,50] The lower-diversity microbiome is reported to be characterized by abundance of pathogenic bacteria such as *Enterococcus*, *Klebsiella*, *Escherichia*, *Staphylococcus* and *Streptococcus*. The single taxonomic unit domination (abundance ≥30%) peaks at 1 week post-transplant, which is followed by a subsequent moderate decrease. The most common dominating taxonomic groups belong to the genera Enterococcus and Streptococcus.[37] Along the same lines, other studies have found the Enterococcus genus to be more prolific during the first month post-transplant, with significantly higher abundance in patients with active or subsequent aGvHD.[51,52] Following allo-HSCT, the microbiome recovery appears to be prolonged and incomplete. In a large cohort of patients (n=753), the post-transplant recovery of the gut microbiota has been reported to start around day +50, but even by day +100 the composition and bacterial abundance observed pre-transplant have not been fully achieved.[53] Moreover, in some patients, microbiota has remained disrupted even 1 year after HSCT, this being particularly the case with butyrate-producing bacteria and *Bifidobacterium*.[54] Eventually, the effect of environmental insult on the intestinal microbiota during allo-HSCT can be so severe that its recovery may require a long time.

Is the disrupted microbiome in allogeneic hematopoietic stem cell transplantation recipients clinically significant?

In the above-mentioned study by Peled *et al.*, reduced microbiome diversity both pre-transplant (days -30 to -6) and peri-engraftment (days +7 to 21), was shown to be significantly associated with lower 2-year OS, while a persistent decrease of this parameter in the latter period was also associated with higher 2-year treatment-related mortality (TRM). Moreover, lower peri-engraftment microbiome diversity in T-cell replete allo-HSCT corresponded to increased GvHD-related mortality, which was not observed in T-cell depleted transplantations. This difference suggests a connection between the microbiota and T-cell alloreactivity.[37] Liu *et al.* revealed a similar association of pre-transplant diversity with mortality as well as a correlation between post-transplant microbiome disruption and acute GI GvHD risk.[55] Furthermore, in a study of 66 patients whose stool specimens were analyzed weekly during the transplantation period up to day +100, Golob *et al.* found a trend of association between near-engraftment low microbiome diversity and the risk for grade 3-4 aGvHD.[56] Likewise, Mancini *et al.* evaluating a cohort of 100 patients, observed a significant connection between low microbiome diversity by day +10 and an increased risk for early (within 30 days) aGvHD.[38]

A number of studies also reported an impact of pre- or post-transplant bacterial abundance on patient outcomes (Table 1). Results of a two-cohort study (a total of 115 adult patients) conducted at the Memorial Sloan Kettering Cancer Center (MSKCC) demonstrated that increased abundance of the genus *Blautia*, including anaerobic commensal bacteria, observed 12 days post-transplant, was associated with reduced GvHD-related mortality and improved OS. At the same time, the use of antibiotics with anti-anaerobic activity and total parenteral nutrition (TPN) correlated with loss of *Blautia*.[57] In the pediatric setting, Biagi *et al.* reported an association of pre-transplant high abundance of *Blautia* and low abundance of *Fusobacterium* with diminished risk for grade 2-4 acute GI GvHD.[58] Additionally, pre-transplant *Enterobacteriaceae* abundance of >5% was associated with an increased risk of BSI and *Lachnospiraceae* abundance of ≤10% appeared to correspond to increased mortality.[38] In a large study from the MSKCC, very high abundance of a bacterial group, mainly composed of *Eubacterium limosum*, in pre-transplant samples or the presence of this group in peri-engraftment samples was found to correspond to a decreased relapse risk,[59] once again emphasizing the association of the microbiome and T-cell immunity. Furthermore, in the study from the Osaka University,[54] *Enterococcus* relative abundance of ≥1% at 1 month post-transplant appeared to be indicative of poor OS, with a 2-year survival of 83.9% for patients with relative abundance of *Enterococcus* <1% *versus* 47.6% for those in whom this parameter was ≥1%. It is noteworthy that none of the surviving patients at 1 year post-transplant displayed *Enterococcus* abundance higher than 1%, sug-

Table 1. Intestine microbial changes in diversity and abundance during pre-transplant and peri-engraftment periods, associated with outcomes of allogeneic hematopoietic stem cell transplantation

Outcome	Pre-transplant	Ref. #	Peri-engraftment	Ref. #
Overall survival ↓	Diversity ↓	37; 55	Diversity ↓	37
			Blautia (day +12) ↓	57
			Enterococcus RA ≥1% (day +30)	54
Transplant- related mortality ↑	*Lachnospiraceae* ≤ 10%	38	Peri-engraftment diversity ↓	37
			Engraftment diversity ↓	40
Acute gastrointestinal GvHD risk ↑	*Blautia ↓	58	≠Diversity ↓	56
	≠Diversity ↓	56	¥Diversity ↓ (day+10)	38
	*Fusobacterium ↑	58	*Lachnospiraceae* (day +10) ↓	38
			Staphylococcaceae (day +10) ↑	38
			Bacteroidaceae ↓	56
			Lachnospiraceae ↓	56
			Enterococcus ↑	52
			# Bacteroides ↑ (at engraftment)	58
GvHD-related mortality ↑			¶ Diversity ↓	37
			Blautia (on day +12) ↓	57
Blood stream infections ↑	*Enterobacteriaceae* (RA > 5%)	38	Enterococcus (RA ≥ 30%) -> VRE ↑	50
			Proteobacteria (RA ≥ 30%) -> GN ↑	50
Relapse ↓	**Eubacterium limosum ↑↑**	59	**Eubacterium limosum presence**	59

↓ represents a decrease in risk; ↑ represents an increase in risk; ↓next to diversity means loss of diversity; ↓ or ↑ next to a bacterial taxa represent decrease or increase in relative abundance, respectively. Different bacterial taxonomic rank is marked as follows: ***phyla*** (***bold and italics***), *family* (*italics*), genus (underlined) and **species** (**bold**). * taxa associated with grade 2-4 acute gastrointestinal graft-*versus*-host disease (GvHD). ≠ diversity associated with grade 3-4 acute GvHD (aGvHD). ¥ diversity associated with early aGvHD, by day 30. #a trend (P=0.05). ¶T-cell replete transplants. RA: relative abundance; VRE: vancomycin-resistant Enterococcus; GN: gram negative.

gesting that this cutoff could serve as a prognosticator of a long-term outcome in this clinical setting.[54] The above evidence suggests that the microbiota changes before and during allo-HSCT are significantly associated with transplant complications and outcomes and might even serve as a predictive marker in this setting.

Can prophylactic fecal microbiota transplantation reduce the risk of infections during allogeneic hematopoietic stem cell transplantation?

In allo-HSCT recipients, curtailment of infection risk is crucial for reducing TRM, particularly due to increased frequency of BSI with multidrug resistant (MDR) bacteria. MDR colonization is established to range between 16% for gram-negative bacteria and 39% for vancomycin-resistant *Enterococcus* (VRE). While BSI have been reported in 16-41% of patients colonized with MDR bacteria, findings regarding a possible association of such colonization with TRM or infection-related mortality are inconclusive.[60-62] In addition, MDR gram-negative colonization has neither been found to correspond to an increased risk for sepsis.[38,63] In the lack of clear evidence, proof-of-concept studies are becoming of increasing importance. Battipaglia *et al.*[64] have evaluated four patients colonized with MDR bacteria who had received FMT on days -46 to -9 before transplant with an aim to limit the risk for infectious complications during HSCT. All the four patients responded with decolonization of the MDR bacteria. One patient developed grade 3 acute gut GvHD on day +30 after transplant (day +51 after FMT) and two others developed bacteremia with sensitive bacteria. Notably, despite receiving broad-spectrum antibiotics during the transplantation period, none of the patients had recolonization of the gut with MDR bacteria.[64] Similar results were reported in a 63-year old HSCT recipient.[65]

The ongoing ODYSSEE trial (clinicaltrials gov. Identifier: 02928523) is aimed at reducing complications that may arise as a result of a loss of microbiota diversity, including infectious complications, poor nutritional status, prolonged hospitalization, as well as therapy discontinuation due to induction treatment-related toxicity in AML patients. Twenty newly diagnosed patients collected pre-induction autologous stools. This autologous FMT was later administered as enema after neutrophil recovery and prior to consolidation chemotherapy. Preliminary results demonstrated safety of this approach, with evidence of stool diversity restoration 10 days after FMT and reduction in antibiotic resistant gene copy count by 43%. Yet, clinical efficacy of this method still needs to be confirmed.[66]

An important pathogen to consider for intervention with FMT is *Clostridium difficile*. The incidence of CDI during allo-HSCT varies between 13% and 30%, mostly in the first month after transplant.[67-69] The disease is usually of mild-to-moderate severity, with good response to treatment; there is no association with TRM, and its possible correlation to subsequent acute GI GvHD is indefinite.[68-70] Given these facts, and the paucity of data on potential efficacy of prophylactic FMT in reducing the risk of CDI among *Clostridium difficile* carriers, FMT prophylaxis may not be required for this indication.

As for the treatment of recurrent CDI, results of three small studies demonstrate safety of FMT administration to a total of 16 patients with recurrent CDI after allo-HSCT, with only three patients recurring after the procedure.[71-73]

Currently available data are insufficient to definitively conclude that prophylactic FMT will reduce the infection rate in the allo-HSCT setting.

Can prophylactic fecal microbiota transplantation reduce the risk of acute graft-*versus*-host disease or transplant-related mortality?

The incidence of clinically significant aGvHD ranges between 22% in allo-HSCT from a matched related donor to 29% in case of a mismatched unrelated donor, with grade 3-4 disease incidence being 8.6% and 12%, respectively.[24] Whether any intervention that restores the microbiome composition could also decrease aGvHD rates is yet to be revealed. Hitherto, only two small studies have reported results of using prophylactic FMT in the post-engraftment period. In the study by Defillip *et al.*,[25] aiming to evaluate safety and feasibility of early restoration of the gut microbiome, frozen capsules of FMT derived from unrelated donors were administered to 13 allo-HSCT recipients 4 weeks after neutrophil engraftment. No FMT-related bacteremia events occurred and two cases of acute GI GvHD were registered. Analysis of stool composition indicated improvement in intestinal microbiome diversity after FMT that was mainly attributed to operational taxonomic units (OTU) originating from the FMT donor.[25] In the study by Taur *et al.*,[53] within 3-28 days of engraftment, patients not receiving broad-spectrum antibiotics, not critically ill and with low abundance of *Bacteroides* (<0.1% of the total 16S sequencing) at that time period, were randomized to either receive autologous FMT (n=14) or to a control group (n=11).

Solely the FMT group was found to reconstitute their microbiome diversity and composition to the pre-transplant state. Of note, the use of autologous FMT raises concern for disrupted microbiota due to prior antibiotic exposure.[53]

These data suggest feasibility and safety of prophylactic FMT; however, its clinical benefit has not been demonstrated yet.

Should additional interventions along with fecal microbiota transplantation aiming to attenuate mircobiome disruption be considered?

Given that a variety of factors could affect the microbiome diversity and composition during the transplantation course, their adequate control might potentially preclude such microbiome changes. The question remains whether FMT alone is sufficient enough or it should be combined with other interventions to provide the required control.

Transplant conditioning

Conditioning chemotherapy itself has a disruptive effect on the microbiome, as found by Montassier *et al.*[26] who evaluated eight lymphoma patients undergoing autologous HSCT with the BEAM (carmustine, etoposide, cytarabine arabine, melphalan) protocol. Since none of the patients received nasogastric tube nutrition, total parenteral nutrition, ciprofloxacin prophylaxis or systemic antibiotic treatment, only the chemotherapy effect on the microbiome was measured. Compared to pre-transplant samples, those drawn at 1 week post-conditioning demonstrated significantly reduced diversity, decreased abundance of *Firmicutes* and *Actinobacteria* and increased presence in bacteroides and proteobacteria, indicating chemotherapy-induced disruption of the intestinal microbiota.[26] Of note, this disruptive effect might be related to etoposide, which has bacterial inhibitory activity.[27,28] Remarkably, the post-transplant decrease in microbiome diversity appeared to be more profound when more intensive conditioning was applied.[74] However, reducing the conditioning intensity was not shown to consistently decrease the rate of aGvHD.[75] Moreover, it might increase the relapse rate and decrease long-term OS.[76,77] Therefore, changing the conditioning regimen in an attempt to attenuate the insult on the microbiome is not currently recommended.

Diet

Dietary interventions such as TPN, prebiotics and probiotics could potentially influence the microbiome composition before or during the transplantation course. TPN administration was reported to be associated with decreased recovery of post-transplant (up to day +120) diversity compared to enteral nutrition. In addition, SCFA levels in the gut content were found to be lower in the TPN group.[78] Iyama *et al.* retrospectively compared a group of patients whose diet was supplemented with prebiotics, i.e., glutamine, fiber and oligosaccharides (GFO) with a group that did not receive such supplementation. GFO was started 7 days before conditioning and continued up to day +28. In the GFO group, duration of diarrhea, mucositis and TPN requirement was shorter and the weight loss was also less prominent.[44] An ongoing prospective trial (clinicaltrials gov. Identifier: 02763033) is evaluating the efficacy of resistant potato starch supplementation between day -7 and day +100 in HSCT recipients. This starch is a non-absorbable carbohydrate that is metabolized by the anaerobic commensal bacteria to produce the SCFA butyrate,[79] shown to reduce the severity of acute GI GvHD in an experimental model.[31] Preliminary results demonstrate the feasibility of this approach in terms of patient compliance, increase in intestinal butyrate levels and abundance of butyrate producing bacteria.[80] As for probiotic supplementation, the available data do not suggest its influence on the microbiome composition or clinical outcomes. It is worth mentioning that the products used in the studies contained only one bacterial strain and not a diversity of bacteria,[43,81] and safety of probiotic administration is of concern in immunocompromised patients.[82]

The loss of diversity during the transplantation course is accompanied with microbiome domination by single taxonomic units such as *Enterococcus*.[37] This enterococcal expansion has been found to be most prominent in patients developing acute GI GvHD.[52] Stein-Thoeringer *et al.* have shown in a gnotobiotic mouse model of allo-HSCT that enterococcal expansion in the gut depends on lactose and its depletion decreases the enterococcal abundance and thus attenuates GvHD severity. Furthermore, in patients with a lactose malabsorption genotype, *Enterococcus* abundance appears to be higher than in patients without this genotype.[83] This finding may give rise to a new approach to dietary intervention during HSCT. Interestingly, in the study by Khandelwal *et al.*, where pediatric allo-HSCT patients under the age of 5 were treated with ready to eat human milk and breast feeding (n=24) or formula (n=14), plasma levels of IL6, IL10, and Reg3α were significantly lower in the group receiving human milk. The microbiome composition also

differed between the two groups, with an increase in pathogenic species such as *E. coli* in the formula-receiving group. Despite the fact that human milk oligosaccharides are metabolized to SCFA by the commensal bacteria, butyrate levels in the stool were similar in both groups. Moreover, no significant difference in the rate of grade 2-4 acute GI GvHD between the groups was revealed. However, the limited size of this study calls for cautious interpretation of these encouraging results.[84] Overall, dietary interventions emerge as a promising way to shape the intestinal microbiota during allo-HSCT. However, results are too preliminary and more research is required before implementing any of these methods.

Antibiotic treatment

The antibiotic treatment applied during the transplantation course is the main factor affecting the microbiome. Quinolone prophylaxis during afebrile neutropenia and systemic broad-spectrum antibiotic treatment with piperacillin-tazobactam or meropenem are widely accepted.[85-87] However, data demonstrate that the use of other antibiotics can better preserve gut beneficial commensals and is associated with improved outcomes.

The study from the University of Regensburg in Germany employed the non-absorbable antibiotic rifaximin and compared it to ciprofloxacin and metronidazole used in a historic cohort of patients for infection prophylaxis during allo-HSCT.[45] Antibiotics were given from day -8 up to engraftment. The urine 3-indoxyl sulfate (3-IS) level was measured as a marker of microbiome diversity.[88] In the rifaximin cohort, the pre-engraftment 3-IS levels were significantly higher without an increase in the sepsis rate or colonization with pathogenic bacteria. This group had significantly lower TRM, prolonged OS and the acute GI GvHD rate tended to be lower in these patients. The observed advantage remained evident even in patients who later received systemic antibiotics for neutropenic fever. [45]

Given the major role of microbiome diversity preservation during allo-HSCT and an association of impaired diversity with acute GI GvHD and adverse patient outcome, Weber *et al.* further compared the effects of various prophylactic and systemic antibiotics in an attempt to identify the ones that could spare commensal bacteria.[89] At 10 days post-transplant, the patient groups receiving rifaximin without systemic antibiotics or rifaximin with systemic antibiotics maintained their microbiome diversity and *Clostridia* abundance and had higher 3-IS levels compared to patients treated with ciprofloxacin/metronidazole ± systemic antibiotics. These results suggest that rifaximin could better preserve microbiome diversity even when systemic broad-spectrum antibiotics are administered during transplantation. Moreover, in the study conducted in two Canadian hospitals and assessing the effect of antibiotic prophylaxis or treatment given before day 0 on frequency of aGvHD and mortality, the authors compared the outcome of a cohort of patients exposed to antibiotics (n=239) to those who did not receive this therapy (n=261).[90] The antibiotic-receiving group demonstrated a significantly higher incidence of grade 2-4 aGvHD and significantly shorter OS at 1, 2 and 10 years post-transplant, indicating an association between the deleterious effect of such treatment on intestinal bacteria and inferior patient outcome.

Importantly, early start of systemic antibiotics (before engraftment) was found to be associated with a lower 3-IS urine level and decreased *Clostridia* abundance in the stool. Furthermore, the TRM rate in such cases was higher than in patients who did not require systemic antibiotics during HSCT or started them after engraftment.[91]

Similarly, systemic treatment with piperacillin-tazobactam and meropenem was reported to correlate with decreased microbiome diversity during the transplantation[37] and significant loss of commensal anaerobic bacteria.[92] In pediatric patients, Simms-Waldrip *et al.*[93] found that higher load of anti-anaerobic antibiotics was associated with a significant decrease in anti-inflammatory *Clostridia* (AIC) abundance, and in patients with aGvHD the abundance decrease was severe (10-log fold) compared to patients without GvHD. In a mouse allo-HSCT model, clindamycin administration was associated with AIC decrease and more severe GvHD, while re-administration of AIC increased its levels in the gut and improved survival.[93] Additionally, Lee *et al.*[94] compared patients who did not require any systemic antibiotic treatment during the transplantation course with those who received cefepime and those who were treated with carbapenem antibiotics. The carbapenem group displayed a significant loss of microbial diversity at engraftment and an increased rate of acute GI GvHD (32.1%) compared to the no-antibiotics group (11.6%). Interestingly, the cefepime group retained a diverse microbiome, demonstrating only a trend to a higher GI GvHD rate (26.4%).

Furthermore, a large multicenter study retrospectively evaluating 857 patients revealed that the use of piperacillin-tazobactam and imipenem-cilastatin was associated with increased 5-year GvHD-related mortality,[95] while this was not observed in patients receiving cefepime and aztreonam. The former antibiotics caused a significant decrease in abundance of *Bacteroidetes* and *Lactobacillus* compared to the latter ones. These results suggest that some antibiotics may be more beneficial than others in the setting of allo-HSCT, and that this beneficial effect is related to the antibiotic ability to be less detrimental to intestinal commensal bacteria.[95] Findings in the pediatric setting were consistent with these data, and exposure to anti-anaerobic antibiotics was reported to result in a significant decrease in butyrate-producing bacteria and the butyrate level in luminal content by day +14. Pediatric patients who later developed aGvHD had a significantly lower butyrate level at that time point than patients without GvHD.[96]

It was also demonstrated that specific antibiotic use during allo-HSCT could change the abundance of specific taxa which was associated with BSI risk. In a cohort of 94 patients, Taur Y *et al.*[50] found that domination of the gut microbiome (abundance ≥30%) by single bacterial taxa *Enterococcus* and *Streptococcus* occurred at the peri-engraftment period (days +10 to +20) in two thirds of the patients. However, treatment with metronidazole increased the risk for enterococcal domination by 3-fold, and this domination elevated the risk for VRE bacteremia by 9-fold. Altogether, these data establish an essential role of antibiotics in disrupting or preserving the intestinal microbiota during allo-HSCT.

Case 1: conclusions

Several issues should be considered in decision-making regarding the appropriate management of this case. This

Table 2. Clinical trials of fecal microbiota transplant in allogeneic hematopoietic stem cell transplantation.

FMT aim	Study (ref.) or ‡NCT number	Number of patients	Outcomes
Prophylactic			
Reduce pathogenic bacteria colonization pre-transplantation			
	Malard *et al.*[66]	20	Restoration of diversity, reduction in antibiotic-resistant gene copy count
	Battipaglia *et al.*[64]	4	All decolonized
	Innes *et al.*[65]	1	All decolonized
Restore microbiome diversity post-transplantation			
	Defillip *et al.*[25]	13	Increase in diversity
	Taur *et al.*[53]	Random: FMT 14 *vs.* control 11	Increase in diversity
Therapeutic			
Recurrent CDI			
	Webb *et al.*[71]	7	No recurrence in 6
	Moss *et al.*[72]	6	No recurrence in 4
	Bluestone *et al.*[73]	3	No recurrence in 1
Steroid refractory/dependent acute GI GvHD			
	Spindelboeck *et al.*[198]	3	2 CR, 1 PR, 3 died
	Kakihana *et al.*[146]	4	3 CR, 1 PR
	Kaito *et al.*[99]	1	CR
	Zhang *et al.*1[100]	1	CR
	Zhong *et al.*[101]	1	CR
	Shouval *et al.*[102]	7	2 CR, 1 PR, 4 died
	Malard *et al.*[103]	8	3 CR, 1 VGPR, 2 PR, 3 died
	Qi *et al.*[104]	8	8 ORR, 2 relapsed, 4 died
	van Lier *et al.*[105]	15	11 CR, 5 relapsed
	Bilinski *et al.*[106]	10	5 ORR, CR 4, SD 1
¶Ongoing clinical trials in GI acute GvHD			
	NCT04269850	¥20	Response and OS
	NCT03819803	¥15	Response
	NCT03812705	¥30	Response
	NCT04285424	¥30	Response
	NCT03359980 (HERACLES trial)	¥32	Response and OS

FMT: fecal microbiota transplant; ‡NCT number: clinicaltrials.gov Identifier. ¶Recruiting or completed from ClinicalTrials.gov; ¥Estimated enrollment; GI: gastrointestinal tract; GvHD: graft-*versus*-host disease; CR: complete remission; PR: partial remission; VGPR: very good partial remission; SD: stable disease; OS: overall survival; ORR: overall response rate.

patient has pre-transplant intestinal microbiota disruption and assumed colonization by MDR bacteria and probably by *Clostridium difficile*. His risk for aGvHD is high, since he has undergone allo-HSCT from a mismatched unrelated donor. Quinolone prophylaxis and meropenem treatment for BSI have further disrupted his intestinal microbiota. The existence of pre-transplant microbiota disruption, mainly attributed to the use of broad-spectrum antibiotics during intensive chemotherapy, is associated with increased TRM, shorter OS and GvHD-related mortality. Pre-transplant FMT can potentially enrich the microbiome diversity and eradicate MDR bacteria or *Clostridium difficile*; however, without controlling such factors as antibiotic prophylaxis and the type of systemic antibiotic therapy employed, the intervention by FMT may not completely achieve its goals.

So far, no data are available regarding a clinical benefit of prophylactic pre-transplant FMT.

While an association between peri-engraftment microbiome low diversity and patient outcome is established, implying potential feasibility of FMT use at that stage, data regarding FMT application before engraftment are not available, and for safety reasons this approach will probably not be attempted. Results of several small-scale studies suggest safety and feasibility of post-engraftment FMT in restoring microbiome diversity (Table 2); however, it remains unknown if this strategy could decrease the risk for aGvHD-related mortality and TRM.

As for dietary interventions at this period, their efficacy is still under investigation. Choosing a different antibiotic prophylaxis, such as rifaximin and systemic antibiotics such as cefepime, looks promising. Nevertheless, new strategies need to be tested to prove their non-inferiority in OS[85] and to establish less disruption for the microbiome (clinicaltrials gov. Identifier: 03078010), especially since fourth-generation cephalosporins have been found in one study to be associated with an increased risk for aGvHD.[97]

Case 1: recommendations

In this case, based on the currently available data, we do not recommend prophylactic administration of pre-transplant or post-engraftment FMT.

Case 2

A 25-year old female with intermediate-risk AML in CR underwent an allo-HSCT with BuCy myeloablative conditioning from her matched sibling. Her neutrophils engrafted by day +14. On day +34 she developed grade 3 aGvHD of the lower GI tract which was steroid refractory (SR). She did not respond to the addition of budesonide, extracorporeal photopheresis (ECP), mofetil mycophenolate or infliximab.

Can fecal microbiota transplantation mitigate prevailing acute gastrointestinal graft-*versus*-host disease?

The current data regarding the use of FMT for the treatment of acute GI GvHD are limited to case reports and small case series (Table 2). A total of 58 described patients were treated with FMT for SR GI grade 2-4 aGvHD. The FMT source was an unrelated donor in 36 cases, a related donor – in six cases and in eight cases a commercial pooled highly diverse FMT was used. FMT was processed and either given fresh within a few hours of collection or it was frozen and later thawed before administration. FMT was administered orally as packed capsules, through a nasogastric/nasoduodenal tube or an enema. Of 58 patients, 28 received FMT after two or more therapy lines, while 19 received it as second-line therapy right after steroid failure. Response was observed in 74% (43 of 58) of patients, with complete response in 57% (33 of 58) and partial response in 17% (10 of 58). Complete response was observed in 73% of patients receiving FMT as second-line therapy. Ten of the responding patients relapsed and 29 patients were alive at the last follow-up (54%; 29 of 54 patients with available data).

Response to treatment was seen within a median of 14 days (range: 3-28), with a median of two FMT (range: 1-7), and a median of 7 days between treatments (range: 2-60).[46,98-106]

Infectious complications occurred in 11 patients. Two had sepsis with bacteria not originating from FMT,[102] and one patient developed diarrhea due to Norovirus that was traced to FMT.[106] Other infections were attributed to the severe immunocompromised state of patients. However, a possible association with FMT could not be ruled out. In responding patients in whom the stool microbiome was sequenced post-FMT, it was found to be significantly more diverse and enriched with *Bacteroides*, *Lactobacillus*, *Bifidobacterium* and *Faecalibacterium* compared to pre-FMT microbiome.[46,98-101] Notably, the diversity increased only upon discontinuation of anti-anaerobic systemic antibiotic treatment, such as piperacillin-tazobactam. However, continuous use or re-initiating treatment with cefepime did not reduce FMT efficiency.[46,98,99]

These results are highly encouraging and support FMT therapy to be relatively safe and effective in SR GI aGvHD.

Case 2: conclusions

Available data suggest a potentially beneficial effect of FMT in acute lower GI GvHD. It should probably be used earlier rather than later, so that patients' response will not be overcome by infectious complications related to extensive immunosuppressive therapy. Discontinuation of antibiotic treatment prior to FMT administration appears to be an important factor contributing to successful response. If antibiotic treatment is required, using cefepime may allow attenuating microbiome insult while maintaining clinical response.

Current information is based on case reports and small series with a wide variability in patient selection, FMT preparation and mode of administration. However, the reported feasibility, safety and clinical benefit appear to be similar across the studies, implying that intestinal microbiota can be recovered with FMT, irrespective of its administration method. Safety remains a concern,[107] especially in advanced GI aGvHD, and if an infectious complication occurs post-FMT, the pathogen should be sequenced and traced to find out if it originates from the FMT.

Case 2: recommendations

Currently, ruxolitinib is the only FDA-approved drug for the treatment of SR aGvHD, while other modalities are also commonly used in this scenario (e.g., extracorporeal photopheresis). Thus, FMT could be recommended for patients with grade 2-4 steroid refractory or dependent aGVHD of the lower GI tract, albeit in the context of a clinical study only.[108-110] Other treatment approaches could also be considered, such as adding it to steroids as part of the first-line therapy (clinicaltrials gov. Identifier: 04269850).

Although clinical trials are still ongoing, given the grave prognosis of SR aGvHD with more than 50% mortality,[111] and the high rate of response to FMT, we recommend considering FMT as a therapeutic option in this setting.

Practical considerations for fecal microbiota transplantation treatment

As FMT has become the standard of care in recurrent and refractory CDI,[112,113] more and more centers are gaining access to FMT programs through either establishing their own stool banks or acquiring FMT from universal stool banks.[114,115]

One of the limiting factors to wider application of stool banks and FMT programs is the lack or variance of regulatory standards. In different countries, FMT is regulated as a drug, tissue or a combined product composed of both human cells and non-human components (microbial DNA and metabolites). Stool banks are recommended to operate under the designated authority in each country. In the absence of local directives, the scientific committee should be responsible for establishing regulatory protocols.[114]

FMT donor screening should follow national regulations and international recommendations.[114] Screening should include medical history related to the risk for transmitting infections, as well as medical conditions and treatments associated with perturbed microbiome (Table 3). Special considerations are to be applied when planning FMT use in allo-HSCT patients, such as testing the donor for Cytomegalovirus and Epstein-Barr virus IgG and IgM, and administering FMT from seronegative donors to seronegative patients. However, when weighing suitability of an FMT donor, one should be cognizant of the fact that no data are available to support the advantage of a particular

donor (a family member, an unrelated donor, or pooled stool from several unrelated donors).

As for autologous FMT, it has not been tested in the setting of aGvHD treatment. Since the microbiota composition of a patient is already disrupted prior to HSCT, using such stool in FMT preparation to be applied for diversity restoration may not be effective. In order to circumvent this problem, in AML patients, we recommend freezing self-stool before the beginning of induction chemotherapy.

In CDI, both fresh and frozen FMT have been shown to be efficient[116] as have been the two delivery routes – colonoscopy and oral capsules.[117] While there are no data pointing to the superiority of either method of preparation or administration for aGvHD treatment, frozen samples from a stool bank allow FMT to be readily available for immediate use without the need to wait for donor screening and FMT collection.

The basic principles of FMT preparation include weighing the sample, suspension in sterile solution (saline), adding glycerol in case the FMT is planned for freezing and storing, homogenization, filtering and aliquoting the suspension for fresh use or freezing (Table 3). The FMT product should be registered and labeled.[114]

Based on the available data (Table 2) we suggest evaluating clinical response at 7-14 days after FMT administration. If no response or only partial response is achieved, we recommend administering a second dose of FMT. Whether in such cases the use of FMT from another donor could provide a superior outcome is yet to be determined. In general, in order to consider FMT as an efficacious therapeutic approach for SR GI aGvHD management, an overall response rate of around 60-70%, with a complete response rate of 30-50% should be a desired target, as these rates are achieved with the use of the approved ruxolitinib treatment and in non-randomized FMT studies.[46,98-106,110]

As for the antibiotic treatment peri-FMT, if feasible, 24-48 hours prior to FMT, systemic antibiotics should be stopped or replaced by one with less anti-anaerobic activity such as rifaximin for prophylaxis or cefepime for febrile neutropenic treatment.[46,98,99]

Microbiome sequencing of donor and patient samples could help interpreting clinical outcomes. It could also be

Table 3. Practical aspects of fecal microbiota transplantation.

FMT stool bank[114]

- Center's own bank
- Acquiring FMT from stool banks of other centers or from a universal stool bank

Regulations[114]

- Set by the designated authority in each country
- Follow international guidelines and recommendations
- If local directives are not available, the center scientific committee should establish regulatory protocols
- FMT for SR GI aGvHD should be given within the setting of a clinical trial

FMT donor screening[114]*

Medical history for infections and risk for infections:

- HIV, hepatitis C, hepatitis B, syphilis, HTLV, other infections, malaria, tuberculosis, illegal drug use, unprotected sex, tissue/organ transplant, recent hospitalization, travel to high risk endemic countries, tattoo, piercing, earing, recent intestinal infection, recent vaccinations with live attenuated virus, blood transfusion, therapy with growth hormone.

Medical history for conditions and medications with risk for microbiota perturbation:

- Chronic gastrointestinal disease (e.g., inflammatory bowel disease, celiac disease), autoimmune disease, cancer, recent GI symptoms (e.g., diarrhea), neurologic disorders, psychiatric disorders, obesity, metabolic syndrome, diabetes, first degree relative with early colon cancer or polyposis. Antibiotic treatment in recent 3 months, chemotherapy, immunotherapy, prolonged use of proton-pump inhibitors, use of probiotics.

Blood tests:

- Hepatitis A, B and C, HTLV, HIV, treponema pallidum, strongyloides stercoralis, NAT for hepatitis B, C and HIV, ANCA (P and C), IgA antibodies level, anti-transglutaminase antibodies, antinuclear antibody, ASCA, liver enzymes, creatinine, calcium, albumin, cholesterol, triglycerides, complete blood count, thyroid function test.

Stool tests:

- Stool culture for *Shigella*, *Salmonella* and *Campylobacter*, direct smear for parasites from different occasions, *Clostridium difficile* antigen, vancomycin-resistant *Enterococci* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae* (CRE), extended spectrum β lactamase producing *Enterobacteriaceae* (ESBL), *Biofire (Biofire FilmArray) multiplex PCR for *Yersinia enterocolitica*, EAEC (Enteroaggregative *E. coli*), EPEC (Enteropathogenic *E. coli*), ETEC (Enterotoxigenic *E. coli*), STEC (Shiga-like toxin producing *E. coli* stx1/stx2), *E. coli* O157, Shigella / EIEC (Enteroinvasive *E. coli*), *Cryptosporidium*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*, Adenovirus F 40/41, Astrovirus, Norovirus, Rotavirus A, Sapovirus, *Campylobacter*, *Clostridium difficile* toxins A and B, *Plesiomonas shigelloides*, *Salmonella*, *Vibrio parahaemolyticus* and *vulnificus*, *Vibrio cholerae*.

Special considerations:

- Cytomegalovirus and Epstein-Barr virus serology (IgM and IgG) when administration to immunocompromised patients is planned.[114]
- Patients with severe food allergy should receive FMT from a donor who will avoid the allergy causing food for 72 hours prior to donation.*
- SARS-CoV-2 screening[120] following the FDA safety alert.[121]

Consent:

- Both donors and patients should sign appropriate informed consent.

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FMT source[122]
- Fresh *versus* frozen: frozen is ready for immediate use.
- Unrelated donor, pooled stool from many unrelated donors, related donor, autologous collected while the patient was still healthy.

FMT preparation and storage[114,122]* (in brief)
- Donor stool collected into a sterile plastic container.
- If not done on site, it should be kept at -4°C until processing.
- Stool processing and storage should be done within 6 hours from collection.
- Processing should be done in a sterile hood
- Weigh the stool (25 g minimum for lower GI FMT and 12.5 g for upper GI FMT).
- Mix with sterile saline, homogenize, filter and centrifuge.
- Re-suspend the pellet with saline.
- If stored frozen, add glycerol to a concentration of 10%.
- Aliquot and label according to way of administration (capsules, tubes)
- Frozen FMT should be kept at -80°C and preferably used within 1 year from collection.

FMT administration[114,122]*

FMT preparation:
- Fresh FMT is given within 6-8 hours from collection.
- Frozen FMT is thawed at 37°C water bath and administered within 4-6 hours.
- Frozen capsules, thawed at room temperature for a few minutes.

Method of administration:
- Upper GI – gastroduodenoscopy, nasogastric tube, nasoduodenal tube, capsules.
- Lower GI - colonoscopy, enema.

Special considerations:
- If possible, to stop antibiotic treatment 24-48 hours prior to administration.
- Or replace current antibiotics with less anti-anaerobic antibiotics (e.g., rifaximin, cefepime)

Monitoring for clinical response in GI aGVHD
- In 7-14 days after administration.
- In case of no response or partial response, consider a second dose.

Stool sampling for later sequencing (16S ribosomal RNA sequencing or other)

From donor:
- A sample from the collected stool of each batch of donation.

From patient:
- A sample obtained before FMT, 1 week, 2 weeks and 4 weeks after FMT, at relapse/progression of GI aGvHD.

Monitoring for adverse events[122]*

Commonly reported:
- Aspiration (in upper GI administration), nausea, vomiting, constipation, diarrhea, bloating, abdominal pain, adverse events caused by the nasogastric tube insertion or colonoscopy procedure, fever.

Infections:
- Diarrhea, colitis, bacteremia, pneumonia.

*National and Institutional guidelines. FMT: fecal microbiota transplantation, SR: steroid refractory, GI: gastrointestinal, aGvHD: acute graft-*versus*-host disease, HIV: human immunodeficiency virus, HTLV: human T-cell leukemia virus, NAT: nucleic acid test, ANCA: anti-neutrophil cytoplasmic antibodies, ASCA: anti- saccharomyces cerevisiae antibodies, CMV: cytomegalovirus, EBV: Epstein-Bar virus.

valuable in distinguishing between the donor and the recipient as the source of post-FMT infection. However, currently there are no data suggesting that patient stool sequencing prior to FMT could guide its administration or affect the outcome. Therefore, given that the primary outcome should be the clinical response to treatment we recommend treating SR GI aGvHD patients with FMT even if the microbiome analysis is not available. Nonetheless, we do suggest storing stool samples from the donor and the patient (before and after FMT) for later sequencing if it becomes available.

Further accumulation of data on FMT for SR GI aGvHD will allow wider and more efficient application of this treatment approach.

Open challenges and future directions

Disruption of the intestinal microbiome during allo-HSCT is a multifaceted process with a cause-and-effect relationship between multiple factors such as conditioning, diet and antibiotic treatment. Lately, FMT has emerged as an intervention that can facilitate microbiome recovery and potentially intervene with the above interplay (Figure 1). The intestinal microbial disruption before and during allo-HSCT is clearly associated with transplant-related outcomes, mainly acute GvHD and mortality, and pre-clinical data demonstrate the key role of the intestinal microbiota in protecting the gut from inflammatory damage and in regulating the innate immune system to maintain a more tolerant state.[118]

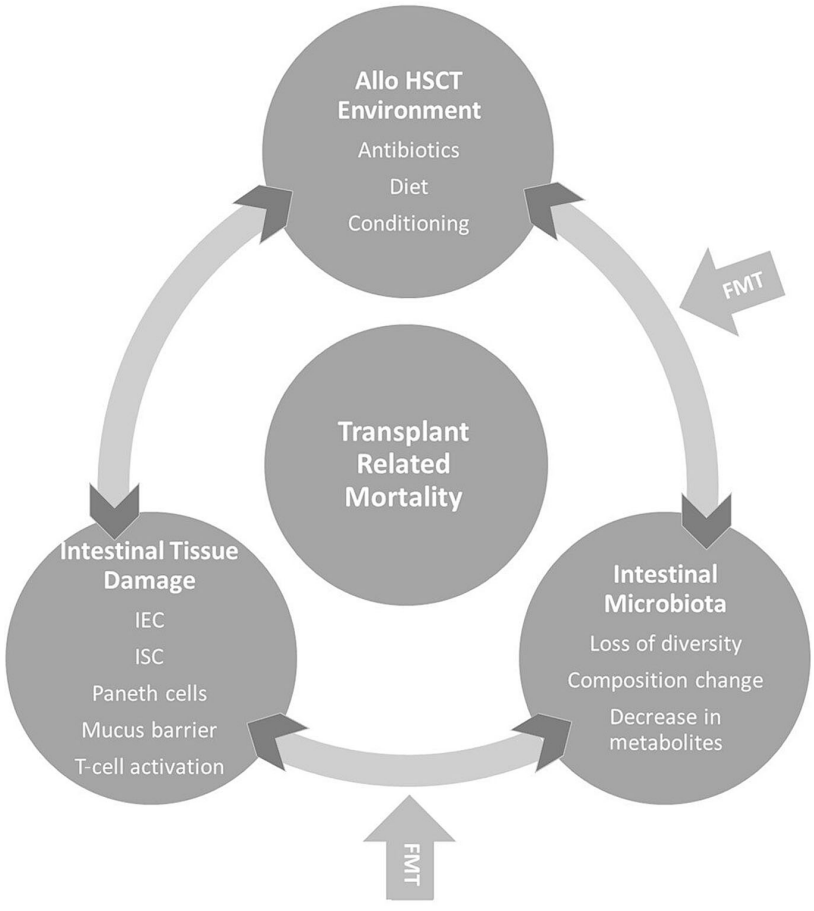

Figure 1. The multifactorial interplay between environmental factors, intestinal microbiota and tissue damage affects transplant-related outcomes. During allogeneic hematopoietic stem cell transplantation (allo-HSCT), conditioning chemotherapy causes damage to the intestinal mucosa cells such as intestinal epithelial cells, intestinal stem cells, paneth cells and mucus producing goblet cells. Gut microbiota is already disrupted before allo-HSCT and due to prophylactic and systemic antibiotic therapy the microbiota disruption worsens with loss of butyrate producing bacteria and other beneficial commensals, along with increase in pathogenic bacteria such as *Enterococcus*. Depletion of bacterial metabolites postpones epithelial cell repair and restoration of the mucus barrier. Pathogenic bacteria can disseminate through the damaged mucosa and cause blood stream infections, which will necessitate the administration of systemic antibiotics further disrupting the intestinal microbiota. This vicious cycle is associated with graft-*versus*-host disease (GvHD), increased mortality and diminished overall survival. The question remains whether fecal microbiota transplantation (FMT) and other interventions such as prebiotics and the use of antibiotics with less anti-anaerobic activity could eventually break the cycle and improve outcomes. IEC:– intestinal epithelial cells; ISC: intestinal stem cells.

While the addition of beneficial bacteria or their metabolites has been shown to ameliorate acute GvHD in animal allo-HSCT models, many challenges remain concerning the role of the intestinal microbiota in allo-HSCT in humans. A substantial amount of basic research is being conducted aiming to better understand the place of microbiome changes in the pathogenesis of acute GvHD. In addition, a large population microbiome analysis is ongoing attempting to delineate the interplay between other factors, such as antibiotics and diet, and the microbiota disruption, and to determine the optimal strategy allowing to preserve the microbiota intact.[119] However, while these issues are still under investigation, clinical trials evaluating the efficacy of FMT and other above-mentioned interventions in the HSCT setting are underway (Table 2). Joint efforts to further explore biological, correlative and recovery functions of the intestinal microbiota could ultimately lead to decreased transplant-related mortality, and even pave the way to personalized therapeutic strategies in HSCT.

Disclosures

No conflicts of interest to disclose.

Contributions

IH, DY-O and TZ wrote the paper.

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