Intestinal Microbiome in Hematopoietic Stem Cell Transplantation For Autoimmune Diseases: Considerations and Perspectives on Behalf of Autoimmune Diseases Working Party (ADWP) of the EBMT

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Over the past decades, hematopoietic stem cell transplantation (HSCT) has been evolving as specific treatment for patients with severe and refractory autoimmune diseases (ADs), where mechanistic studies have provided evidence for a profound immune renewal facilitating the observed beneficial responses. The intestinal microbiome plays an important role in host physiology including shaping the immune repertoire. The relationships between intestinal microbiota composition and outcomes after HSCT for hematologic diseases have been identified, particularly for predicting the mortality from infectious and non-infectious causes. Furthermore, therapeutic manipulations of the gut microbiota, such as fecal microbiota transplant (FMT), have emerged as promising therapeutic approaches for restoring the functional and anatomical integrity of the intestinal microbiota post-transplantation. Although changes in the intestinal microbiome have been linked to various ADs, studies investigating the effect of...
intestinal dysbiosis on HSCT outcomes for ADs are scarce and require further attention. Herein, we describe some of the landmark microbiome studies in HSCT recipients and patients with chronic ADs, and discuss the challenges and opportunities of microbiome research for diagnostic and therapeutic purposes in the context of HSCT for ADs.

Keywords: autoimmune diseases, autoimmunity, fecal transplantation, intestinal, microbiome, stem cell transplantation, HSCT = hematopoietic stem cell transplant

INTRODUCTION

Intestinal microbiota may positively affect many aspects of the host physiology, including absorption of nutrients, prevention of overgrowth by potential pathogens, maintenance of epithelial barrier function, and shaping the immune system (1). Studies of the microbiome in the setting of hematopoietic stem cell transplantation (HSCT) demonstrated that intestinal flora are of particular importance in determining treatment outcomes, influencing immune reconstitution, and impacting complications such as infections or graft-versus-host disease (GvHD) (2, 3). In addition, changes in the microbial composition and function have been associated with various autoimmune diseases (ADs), and, although the precise mechanistic links between the microbiome and ADs remain largely unknown, increasing evidence suggests that disturbed gut microbiota contribute to pathogenesis (4). Among the potential mechanisms in the complex interplay between gut microbiota and host immune system, abnormal microbial translocation, molecular mimicry, and dysregulation of local and systemic immunity have been postulated.

This article will summarize the current evidence supporting the relationship between the microbiome and specific ADs, its impact on transplant outcomes, and potential therapeutic interventions, such as fecal microbiota transplantation (FMT). Moving forward, we propose how we may evaluate and influence the microbiome in the setting of HSCT for ADs to affect immune reconstitution and potentially improve clinical outcomes.

INTERACTION BETWEEN GUT MICROBIOTA AND THE HOST IMMUNE SYSTEM

While the primary function of the intestinal microbiota for the host has been considered to be the digestion of complex sugars and the provision of essential vitamins, it has become clear that the microbiota play an important role in the education and shaping of a functioning immune system. Evidence for this comes from the analysis of germ-free mice, which in the absence of any microbiota have underdeveloped lymph organs and reduced innate immune competence resulting in increased susceptibility to infection (5). Most likely for similar reasons, germ-free mice are resistant to genetic and induced models of autoimmunity. While the molecular mechanisms are still poorly understood, several pathways involved in the microbiota–host interaction have been identified, ranging from provision of ligands for innate receptors, such as Toll-like receptors for “trained” immunity (6), to the production of short-chain fatty acids, a product of the metabolizing of dietary fibers by certain bacteria, which have been described to enhance immune regulation (7, 8). Reciprocally, the host controls the microbiota through the production of antimicrobial peptides by intestinal epithelial cells and copious amounts of IgA antibodies, which are actively transported into the gut lumen by the intestinal epithelial cells, controlling the growth, mobility and attachment of intestinal bacteria (9). Alterations to this intricate microbiota – host interaction, e.g. genetic defects disrupting microbial sensing of the host or loss of bacterial diversity, often summarized under the term dysbiosis, resulting in loss of microbial functions for the host, has been associated with the development of chronic inflammatory diseases (10). Mechanistically, several pathways have been discussed by which intestinal microbiota might contribute to the development or perpetuation of autoimmune diseases (11). They include gut dysbiosis, which disrupts local gut homeostasis and may promote translocation of commensal or pathobions to tissues where they facilitate chronic inflammation. In addition, microbiota may trigger autoimmunity directly by providing antigenic stimuli resulting in cross-reactivity of autoreactive lymphocytes and autoantibodies with bacterial orthologues. Finally, microbiota may modulate the immune system through their metabolites and may facilitate immune regulation by stimulating regulatory immune elements (summarized in Figure 1).

MICROBIAL PROFILING

The introduction of molecular biological methods for the characterization of the microbiota, in particular high-throughput sequencing, has greatly advanced our understanding of the diversity and function of the microbiota (22). Sequencing of the single or a combination of the 9 variable regions of the gene for the 16S ribosomal RNA of the small 30S ribosomal subunit is the mainstay to describe the composition of a microbial community. While 16S rRNA sequencing has become the method of choice due to its simplicity, it is often limited in the taxonomic resolution and is prone to bias e.g. PCR amplification and sampling depth (23). More extensive sequencing approaches include whole 16S rRNA gene sequencing allowing resolution of the microbiome to the species level, however often at the cost of sampling depth, and shot-gun metagenomics sequencing which will additionally yield information on the genetic repertoire, i.e. potential functional genes, of the bacterial community, the latter requiring
extensive bioinformatics resources. Other “omics”, such as metaproteomics can also be used to define the composition as well as the function of the microbiota, while metabolomic profiling identifies the mediators with which the microbiota could interact within itself and with the host [reviewed in (24)]. Recently, the combination of absolute quantification of the microbiota by flow cytometry with 16S rRNA gene profiling was shown to better reflect clinically relevant changes of the microbiome in patients with inflammatory bowel diseases (IBD) (25). Flow cytometric analysis of the microbiota also has the potential to rapidly identify alterations in the microbiota on the single cell level for monitoring purposes (26) and when combined with cell sorting and 16S rRNA gene analysis could lead to the identification of relevant bacteria in a more targeted fashion.

ROLE OF INTESTINAL MICROBIOTA IN AUTOIMMUNE DISEASES

Inflammatory Bowel Diseases

The intestinal tract, home to the largest density and diversity of microorganisms in healthy humans, is the target organ of IBD comprising Crohn’s disease (CD) and Ulcerative Colitis (UC). The chronic intestinal inflammation in IBD is characterized by effector and tissue resident memory T cell responses to aspects of the intestinal microbiota (27). Despite large inter-individual variability, genetic analyses of microbial populations in stool and/or mucosal biopsies has revealed an overall decrease in diversity, a loss of symbionts and an increase in pathobionts (essentially Gram-negative proinflammatory microbes) in both UC and CD (27, 28). Whether these changes in microbial composition and IBD pathogenesis are a cause or consequence of intestinal inflammation remains a key area of study (29). Alterations in gut microbiota can disrupt epithelial and immune homeostasis, leading to increased permeability and eventual immune activation. Alternatively, the documented genetic and/or microbial-independent environmental factors associated with IBD may promote inflammation and oxidative stress, which subsequently results in a shift in microbial composition. A recent study has shown that increased fecal proteolytic activity and microbiota changes precede diagnoses of ulcerative colitis (30). In addition, the altered humoral and cellular acquired immune responses towards bacterial antigens that characterize IBD, particularly Crohn’s disease (31), may predate disease onset (32). This suggests that immune responses towards microbes, rather than microbial composition itself, drives epithelial barrier disruption and altered innate responses at disease onset. Thus, in marked contrast to the impressive efficacy seen in Clostridium difficile infection (33), FMT has shown some benefit in mild UC but no impact in CD (34–36). Nonetheless, regardless of whether dysbiosis is the initial event or the result of overt inflammation, shifts in microbial composition may help perpetuate disease, as well as impact response to therapy in IBD (37, 38), and thus represent a desirable target for future therapies.

Systemic Sclerosis

In systemic sclerosis (SSc), a rare systemic autoimmune disease characterized by vasculopathy, immune activation and consequent progressive fibrosis, multiple genetic, epigenetic, and environmental factors are regarded as potential triggers for the onset and progression of the disease (39). Over the past decades, emerging evidence suggests that alterations of microbial populations colonizing epithelial surfaces (i.e., gastrointestinal tract, skin and lung), known as dysbiosis, may contribute to chronic inflammation and autoimmunity (4). Since the gastrointestinal tract is one of the organs highly affected in SSc, recent studies have aimed to investigate gastrointestinal microbiota alterations to elucidate the possible interaction with disease phenotype and clinical outcome of the disease (40).
Initial studies found that specific bacteria, particularly beneficial commensal genera (Faecalibacterium, Clostridium and Rikenella) and, conversely, more potentially pathobiont genera (Bifidobacterium, Fusobacterium and Prevotella) were decreased in SSc patients compared to healthy controls (41–43). Notably, SSc patients with more severe gastrointestinal symptoms exhibited a prevalence of the pathobiont Fusobacterium compared to patients with mild or no gastrointestinal symptoms (41–43). Furthermore, overabundance of opportunistic pathogenic Clostridium and typically oral Streptococcus species was recently described in SSc, while Alistipes, Bacteroides, and butyrate-producing species were depleted, congruent with findings in patients with IgG4-related disease, suggesting a common signature in both fibrosis-prone autoimmune diseases (44). Altogether, these studies confirm the existence of a shift in gut microbiota population in SSc patients. Whether these changes are causative or rather reflect the gastrointestinal involvement by inflammatory and fibrotic processes remains to be demonstrated. The role of intestinal dysbiosis in the disease pathogenesis is further complicated by the possibility that, as showed in other diseases, the intestinal microbiota in SSc might modulate local immunological mechanisms possibly responsible of local and systemic alterations (45).

Multiple Sclerosis
Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS), which results from interactions of genetic and environmental factors (46). The underlying pathological process is complex but includes the abnormal activation of T and B cells targeting foreign and/or self-antigens, which could be primed within the CNS or in the periphery (47, 48). A potential source of such antigens is the gut microbiome, which exhibits a level of homology to human myelin proteins and may trigger cross-reactivity through the mechanism of ‘molecular mimicry’ (49, 50). Immune reconstitution studies have shown that gut Mucosal Associated Invariant T (MAIT) cells, which express chemokine receptor 6 (CCR6) to facilitate their transmigration into the CNS, are reduced following autologous HSCT, suggesting that they may play a role in crosslinking gut microbiome with the neuroaxis (51). In one experimental model, the presence of intestinal microbiota was necessary to induce CNS autoimmunity, suggesting that the gut has the ability to control systemic autoimmune responses (52, 53). Germ-free mice recipients receiving feces from patients with MS develop severe Experimental Allergic Encephalomyelitis (EAE) and the presence of intestinal microbiota in SSc might modulate local immunological mechanisms possibly responsible of local and systemic alterations (45).

ROLE OF INTESTINAL MICROBIOTA IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

Correlation With HSCT Outcomes
The intestinal microbiome undergoes profound changes during the course of transplantation. Multiple transplant-related factors (i.e. conditioning regimen, broad-spectrum antibiotics, nutrition) drive microbial shifts. At the same time, the alteration in the composition of gut flora is associated with transplant outcomes, including overall survival (OS), progression-free survival (PFS), treatment-related mortality (TRM) and GvHD (Table 1). Bacterial diversity largely decreases after HSCT, and is correlated with increased risk of major transplant complications such as infections or GvHD, potentially affecting the outcome of the procedure (81, 82). A large multicenter observational study has confirmed lower mortality rates in patients showing higher diversity of intestinal microbiota at engraftment (5). Recently, microbiota injury has been observed also in recipients of autologous HSCT, who undergo similar antibiotic exposures and nutritional alterations after high-dose chemotherapy and transplant procedure (80). Reduced OS and PFS have been reported in patients with lower peri-engraftment microbiome diversity.

Impact of chemotherapy, Diet, and Antibiotics on the Intestinal Microbiome in Transplant Recipients
Microbiome and transplant correlations may be influenced by local practices, antibiotic choices, hospital flora, and diet. Gastrointestinal disturbances associated with chemotherapy and radiation (83) and subsequent mucositis can also impact the composition of intestinal microbiota. A reduction in α-diversity and significant differences in the composition of the intestinal microbiota have been observed in response to chemotherapy, such as increase in Bacteroides and Enterobacteriaceae paralleled by a decrease in Bifidobacterium, Faecalibacterium prausnitzii, and Clostridium cluster XIVa (84), and a drastic drop in Faecalibacterium accompanied by an increase of Escherichia (85). The impact of diet on gut flora is well-recognized (86). Depletion of the intestinal microbiota reduces visceral adipose tissue and caloric uptake from diet (87), and enteral feeding may exert a beneficial effect on intestinal flora by providing the required nutrients (88). Interestingly, a lactose-free diet can prevent microbial overdominance by detrimental commensal bacteria like Enterococcus (72). Broad-spectrum antibiotic prophylaxis/treatment, commonly used in HSCT recipients, in the early phase after HSCT can beneficially reduce the number of transmigrated bacteria. However, their long-term effects are detrimental, because they limit microbiota diversity, by killing beneficial commensal bacteria that inhibit pathogens and promote immune defenses (81). A drastic decrease in the diversity of enteric microbiome after administration of antibiotic therapy, and the loss of obligate anaerobic commensal...
TABLE 1 | Impact of microbiome on HSCT outcomes.

| Study | Study Population | Microbiome Analysis | Microbiome Biomarker | HSCT Outcome |
|-------|------------------|---------------------|----------------------|--------------|
| Taur et al., 2012 (69) | 94 adult patients | 454 pyrosequencing, V1-V3 region of the 16S | Enterococcus domination (>30%) | VRE Bacteremia 9-fold increased risk |
| | Allogeneic HSCT | RNA gene | Proteobacteria domination (>30%) | Gram negative Bacteremia 5-fold increased risk |
| | Single center, USA | | | |
| Ubeda et al., 2013 (66) | 94 adult patients | 454 pyrosequencing, V1-V3 region of the 16S | Bacteroidetes domination (>30%) | Protection from VRE domination |
| | Allogeneic HSCT | RNA gene | | |
| | Single center, USA | | | |
| Taur et al., 2014 (61) | 80 adult patients | 454 pyrosequencing, V1-V3 region of the 16S | Low bacterial diversity at engraftment | Lower OS |
| | Allogeneic HSCT | RNA gene | | |
| | Single center, USA | | | |
| Holter et al., 2014 (62) | 31 adult patients | Roche 454 platform sequencing, V3 region of the 16S RNA gene | Enterococcus abundance > 20% | Increased frequency of GI acute GvHD |
| | Allogeneic HSCT | | Urinary indoxyl sulfate levels decrease during aplasia after HSCT | |
| | Single center, Germany | Strain-specific PCR of enterococci | Urinary indoxyl sulfate analysis ** | |
| Weber et al., 2015 (63) | 131 adult patients | Roche 454 platform sequencing, V3 region of the 16S RNA gene | Low urinary indoxyl sulfate levels within 10 day after HSCT | Low OS |
| | Allogeneic HSCT | | | High TRM |
| | Single center, Germany | Strain-specific PCR of enterococci | Urinary indoxyl sulfate analysis ** | |
| Jenq et al., 2015 (64) | 115 adult patients | First cohort (n=64): Roche 454 platform sequencing, V1-V3 region of the 16S RNA gene | Increased bacterial diversity | Higher OS |
| | Allogeneic HSCT | | Low bacterial diversity | Lower TRM |
| | Single center, USA | Second cohort (n=51): Illumina MiSeq platform sequencing, V4-V5 region of the 16S RNA gene | Blautia genus # abundance | Lower GvHD related mortality |
| Shono et al., 2016 (65) | 857 adult patients | Illumina MiSeq platform sequencing, V4-V5 region of the 16S RNA gene | Abundance of Eubacterium limosum and other related bacteria | Lower OS |
| | Allogeneic HSCT | | | Higher risk of microbiologically confirmed sepsis, severe sepsis and septic shock |
| | Single center, USA | | Basal Lachnospiraceae: >5% | |
| Harris et al., 2016 (66) | 94 adult patients | 454 pyrosequencing, V1-V3 region of the 16S | Low baseline diversity | Higher GvHD related mortality |
| | Allogeneic HSCT | RNA gene | Enterococcus domination (>30%) | Higher grades 2-4 acute GvHD |
| | Single center, USA | | γ-Proteobacteria domination (>30%) | Higher GII acute GvHD |
| Peled et al., 2017 (67) | 541 adult patients | Illumina MiSeq platform sequencing, V4-V5 region of the 16S RNA gene | Abundance of Eubacterium limosum and other related bacteria | Higher risk of pre-engraftment pulmonary complications |
| | Allogeneic HSCT | | | Higher risk of post-engraftment pulmonary complications |
| | Single center, USA | | | |
| Mancini et al., 2017 (68) | 96 adult patients | Roche 454 platform sequencing, V3-V5 region of the 16S RNA gene | Baseline Enterobacteriaceae: >5% | Lower relapse/progression of disease risk |
| | Allogeneic HSCT | | | |
| | Single center, Italy | Baseline Lachnospiraceae: ≤10% | Baseline Lachnospiraceae: ≤10% | |
| Doki et al., 2017 (69) | 107 adult patients | Roche 454 platform sequencing, V1-2 region of the 16S RNA gene | Higher abundance of Firmicutes, lower abundance of Bacteroidetes, higher abundance fecal bacterium and Eubacterium at baseline | Higher risk of acute GvHD |
| | Allogeneic HSCT | | | |
| | Single center, Japan | | | |
| Lee et al., 2017 (70) | 234 adult patients | Illumina MiSeq platform sequencing, V4-V5 region of the 16S RNA gene | Combined abundance of Bacteroidetes phylum, Lachnospiraceae family, Ruminococcaceae family, Enterobacteriaceae at various rank designations | Protection from Clostridium difficile infection |
| | Allogeneic HSCT | | | Higher risk of Clostridium difficile infection |
| | Single center, USA | | | Higher risk of acute GvHD |
| Golob et al., 2017 (71) | 66 adult patients | Illumina MiSeq platform sequencing, V3-V4 region of the 16S RNA gene | Presence of oral Actinobacteria and oral Firmicutes in stool, deficit of Lachnospiraceae at neutrophil engraftment | |
| | Allogeneic HSCT | | | |
| | Single center, USA | | | |
| Stein et al., 2017 (72) | 1325 adult patients | Illumina MiSeq platform sequencing, V4-V5 region of the 16S RNA gene | Enterococcus domination (>30%) at early post-transplant period | Lower OS |
| | Allogeneic HSCT | | (day 0 to day +12) | Higher GvHD related mortality |
| | Single center, USA | | | Higher grades 2-4 acute GvHD incidence |
| Theisinger et al., 2019 (73) | 72 adult patients | Illumina MiSeq platform sequencing, V4-V5 region of the 16S RNA gene | Low microbial diversity at engraftment | Higher risk of intestinal acute GvHD |
| | Allogeneic HSCT | | | Higher TRM |
| | Single center, USA | | | Higher risk of intestinal acute GvHD |
| Galowsky-Petka et al., 2019 (74) | 44 adult patients | Illumina MiSeq platform sequencing, V4 region of the 16S RNA gene | Low Conibacteriaceae at engraftment | Lower acute GvHD risk |
| | Allogeneic HSCT | | | Higher severe GI acute GvHD risk |
| Biagi et al., 2019 (75) | 36 pediatric patients | Illumina MiSeq platform sequencing, V3-V4 region of the 16S RNA gene | Enterococcus faecalis at various rank designations | Higher grades 2-4 acute GvHD risk |
| | Allogeneic HSCT | | | |
| | Four centers, Italy | Baseline Enterobacteriaceae: >5% | Baseline Lachnospiraceae: ≤10% | |

(Continued)
bacteria such as *Clostridia* and *Bacteroidetes* after piperacillin-tazobactam and meropenem administration, are recurrent in literature (89). Metronidazole administration increases enterococcal domination, whereas fluoroquinolone administration reduces domination by *Proteobacteria* (59) and represents an important variable associated with overall survival (61). Broad spectrum antibiotics, by inducing loss of bacterial diversity, are also associated with increased GvHD-related mortality (65, 90).

**Intestinal Microbiome, Immune Reconstitution, and Infection Prevention**

Effective and appropriate immune reconstitution is central to successful HSCT. Microbiota populations may influence immune reconstitution and cell dynamics in humans (91). The depletion of the intestinal microbiota impairs post-transplant immune reconstitution (87). Analysis of daily changes in circulating immune cell counts and extended longitudinal
microbiota analysis revealed consistent associations between gut bacteria and immune cell dynamics, paving the way for potential microbiota-targeted interventions to improve immunotherapy and treatments for immune-mediated diseases (91).

The gut microbiota play a critical role in maintaining colonization resistance against intestinal pathogens, thus preventing infections. Domination by *Enterococcus* and *Proteobacteria* are associated with the risk of bacteremia by Vancomycin-resistant *Enterococcus* and gram-negative rod respectively (59). A different baseline distribution of the gut microbiome (68) has been reported in patients at risk for microbiologically confirmed infection (high level of *Enterobacteriaceae*, low level of *Lachnospiraceae*), sepsis and septic shock (high level of *Enterobacteriaceae*). Moreover, a documented bloodstream infection may be anticipated by expansion and dominance of pathogenic strains in the gut flora (59, 68, 92, 93). Overall, a low diversity of the intestinal microbiota at engraftment has been shown to be an independent predictor of TRM from both infectious and non-infectious causes (61).

**Intestinal Microbiome, GvHD, and Immunosurveillance**

In the allogeneic transplant setting, a regulatory effect of the gut microbiota in the maintenance of intestinal homeostasis has been reported (94). Loss of fecal diversity, as well as increased abundance of members from *Enterococcus* or *Staphylococcus* species have been associated with the incidence and severity of acute GvHD (79), while other organisms such as *Blaunia* species have a protective role (64). Metabolites produced by intestinal bacteria may promote intestinal tissue homeostasis and immune tolerance in the context of acute GvHD (95). Moreover, commensal bacteria can also play a role in tumor immunosurveillance. Increased abundance of a cluster of related bacteria including *Eubacterium limosum* was associated with decreased risk of relapse or disease-progression (67).

Altogether, these results indicate that the intestinal microbiota represent a potentially important factor in the success or failure of HSCT. As such, the microbiome can be envisioned both as a biomarker for the identification of patients at higher risk for transplant-related complications, and also a target for intervention aiming to impact clinical outcomes through enhancing microbiota recovery (96).

**Modulation of Gut Microbiota by Fecal Microbiota Transplantation**

FMT is a recommended therapeutic strategy for treating recurrent *Clostridoides difficile* infection (97, 98). Additionally, FMT has been investigated for treatment of steroid-resistant acute GvHD and initial positive results (99) were confirmed by several case reports (100). A small cohort study recently reported a complete response in 10 out of 14 patients (71%) with steroid-refractory or steroid-dependent acute GvHD 28 days after FMT (101). This response was accompanied by an increase in microbial α-diversity, a partial engraftment of donor bacterial species, and increased abundance of butyrate-producing bacteria, including groups in the order *Clostridiales*, namely *Blautia* species. Malard et al. recently reported the use of a next-generation FMT product “MaaT013”, a standardized, pooled-donor, high-richness microbiota biotherapeutic, in the largest cohort of patients to date (n=29) with steroid-refractory or steroid-dependent intestinal acute GvHD (102). These patients had previously received and failed 1 to 5 lines of GvHD systemic treatments. The product was well tolerated and at day 28, overall response and complete remission rates were 59% and 31%, respectively. Furthermore, some studies have evaluated the role of FMT in treating dysbiosis after allogeneic HSCT. Taur et al. reported that autologous FMT after HSCT was safe and boosted microbial diversity, restoring bacterial populations lost during HSCT and reversing the disruptive effects of the broad-spectrum antibiotics (n=14) (81). Overall, FMT appears to be a promising strategy and several studies are ongoing to evaluate FMT for acute GvHD management (NCT03812705, NCT03492502, NCT03359980, NCT03720392, NCT03678493). Regarding prevention of complications, additional studies are warranted to confirm that restoration of gut microbiota dysbiosis after FMT translates into clinical improvement after allogeneic HSCT, in particular a lower incidence of acute GvHD (96).

**DISCUSSION**

It is increasingly accepted that understanding the complex interactions between the microbiome and immune system will be crucial to defining the pathogenesis of ADs, whilst optimizing therapeutic interventions and clinical outcomes. HSCT is increasingly used specifically to treat severe, resistant ADs, with now more than 3000 cases being reported to the registry of the European Society of Bone and Marrow Transplantation (EBMT) (103, 104). To date very limited data is available regarding microbiome biology in the setting of HSCT for ADs, where medium to long-term clinical outcomes are considered to be due to the induction of altered (or ‘re-booted’) immune reconstitution post-transplant. The ‘immune re-boot’ has been increasingly characterized in a range of ADs with a range of immunological markers, including evidence of generation of ‘re-educated’ and regulatory populations to support re-induction of self-tolerance lasting beyond the broad immunosuppressive effects of autologous HSCT (105, 106). Changes in immune reconstitution may affect not only on disease activity, but also adverse events, such as secondary ADs (107–110).

As for ADs outside the transplant setting, and for GvHD in allogeneic HSCT, the microbiome may significantly influence the baseline status of the underlying AD pre-transplant, the patients general condition peri-transplant (which will inevitably be influenced by the treatment and supportive care, especially antibiotics), and then the dynamics of the reconstituting immune system post-transplant. The microbiome may therefore influence short- and long-term immune recovery and clinical outcomes following autologous HSCT. Therefore, future
investigations evaluating microbiome changes pre-, peri- and post-HSCT in ADs patients are warranted. Table 2 includes proposed recommendations for studies of the microbiome (111–115) that could be compared with clinical outcome and laboratory data related to immune reconstitution in patients undergoing HSCT for various ADs. Although bio-banking and testing cannot be regarded as routine care, they could be integrated into clinical trials or observational studies with appropriate institutional approvals. In future, a greater understanding may help design of prospective studies of interventions, including FMT, to test the proof of principle of modulation of the microbiome in this setting.

In conclusion, we have summarized the current evidence supporting the relationship between the microbiome, HSCT and ADs, and speculated on the potential impact of the microbiome on clinical outcomes and immune reconstitution following HSCT for severe, resistant ADs. The evidence in this specific field is currently very limited, warranting harmonization of the microbiome monitoring and prospective studies to evaluate properly any potential impact and/or clinical benefit.

### DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article-supplementary material. Further inquiries can be directed to the corresponding authors.

### AUTHOR CONTRIBUTIONS

TA, RG, and JS led on concept and design. TA and RG led on coordination and data analysis, provided expert and analytical feedback and were involved in reviewing, writing and editing the manuscript. All authors contributed to the analysis and interpretation of data, and writing sections of the manuscript. The experts on this panel are active members of the EBMT. All co-authors were involved in drafting the paper, revising it critically, and approval of the submitted and final versions.

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