



Mucosal microbiotas and their role in stem cell transplantation

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Mucosal microbiotas and their role in stem cell transplantation. Patients with hematological disorders such as leukemia often undergo allogeneic hematopoietic stem cell transplantation, and thereby receive stem cells from a donor for curative of disease. This procedure also involves immunosuppressive and antimicrobial treatments that disturb the important interactions between the microbiota and the immune system, especially at mucosal sites. After transplantation, bacterial diversity decreases together with a depletion of Clostridia, and shifts toward predominance of Proteobacteria. Infectious and inflammatory complications, such as graft-versus-host disease, also interfere with patient recovery. This review collects and contextualizes current knowledge of the role of mucosal microbiotas at different body sites in stem cell transplantation, proposes underlying mechanisms, and discusses potential clinical value of bacterial markers for improved treatment strategies.

Key words: Antibiotics; blood cancer; chemotherapy; Clostridia; graft-versus-host disease; hematopoietic stem cell transplantation; microbiome; microbiota; mucosal immunity; Proteobacteria.

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THE MUCOSAL MICROBIOTA

In the intestine, as well as the oral and nasal cavities, mucosal tissues constitute the first line of defense against invading pathogens, facilitated by mutual interactions of commensal microbial communities and the host immune system at the mucosal surfaces [1–3]. The intestinal mucosal barrier consists of a single layer of epithelial cells linked by tight junctions and is located between a mucus layer and the lamina propria. Besides nutrient absorption, the major function of the epithelial cells is to prevent translocation of potential pathogens [2]. Within the oral cavity, the environment of the buccal mucosa is unique as it is directly interacting with the innate and the adaptive immune system, similar to the intestinal mucosa [4]. Among the different niches within the nasal cavity, the anterior nares are most exposed to external influences. The

epithelium lining the anterior nares resembles that of the skin and is lined with sebaceous glands and coarse hairs [4].

As part of the innate immune system, antimicrobial peptides (AMPs), produced by, for example, Paneth cells and epithelial cells, shape the commensal microbiota and form an important chemical barrier toward invading pathogens [5]. The microbiota also contributes to AMP-mediated innate immune function: For instance, the expression of regenerating islet-derived protein III-gamma (RegIII γ), an AMP produced by Paneth cells and intestinal epithelial cells, is dependent on commensal microbial toll-like receptor-mediated stimulation [6,7].

The adaptive immune system is a key player in maintaining host-microbial homeostasis, characterized by a diverse microbiota, integrity of mucosal barrier function, and minimal inflammation [2,8]. For instance, mucosal colonizers produce short-chain fatty acids (SCFA), which mediate IgA

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production by plasma cells. In turn, IgA is an important regulator of the microbiota, which coats both commensals and pathogens, promoting targeted effector cell reactions and preventing bacterial invasion of deeper mucus layers [2,8].

Of no less importance are interrelations of the microbiota and the cell-mediated part of the adaptive immune system. In mice, specific commensals, such as segmented filamentous bacteria, *Akkermansia muciniphila* and taxa affiliated with Clostridia and *Bacteroides*, can activate T cells, especially the CD4+ T cell subpopulations of T helper 17 (TH17) cells and T regulatory (Treg) cells [8,9]. By activating TH17 cells, the microbiota promotes mucosal integrity since TH17 cells protect against extracellular bacterial and fungal pathogens, for example, in the gut and the oral cavity [10]. However, upon high cytokine stimulation, TH17 cells can promote inflammation that requires anti-inflammatory counter-action by immunosuppressive Treg cells [2,8]. Expansion of Treg cells underlies commensal microbial influence, as well. For instance, several *Bacteroides* and Clostridia strains originating from human feces were shown to induce Treg cells in mice, for example, through SCFA and polysaccharide A production [2,11–13].

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT)

In allogeneic hematopoietic stem cell transplantation (allo-HSCT), the patient is infused with stem cells derived from a donor to cure a malignant or non-malignant hematological disorder, such as blood cancer [14]. The immunotherapeutic aims of allo-HSCT are to restore the recipient's depleted hematopoietic system and to achieve a graft-versus-leukemia (GvL) effect of donor-derived cells against malignant cells in the recipient [14]. To eradicate the cancer cells and reduce the risk of graft rejection, recipients undergo a preparative myeloablative conditioning regimen with high-dose cytotoxic chemotherapy, sometimes in combination with total body irradiation (TBI), before stem cell transplantation [14] (Fig. 1). Acute graft-versus-host disease (aGvHD) is a major complication and one of the main causes of death following allo-HSCT [15]. In aGvHD, immunocompetent donor-derived T cells attack healthy recipient tissue [15].

Infectious complications contribute decisively to the morbidity and mortality after allo-HSCT [16]. Inherent characteristics of allo-HSCT, including myeloablation (i.e., suppression of the bone marrow's production of blood cells), immunosuppression, and subsequent delayed immune

reconstitution, are major risk factors for infections [16]. Therefore, patients usually receive prophylactic antimicrobial treatment during conditioning prior to allo-HSCT (Fig. 1).

The procedure of hematopoietic stem cell transplantation and its accompanying treatments interfere in various ways with crucial host-microbial interactions at multiple body sites. Within this process, disturbance of host-microbiota homeostasis is introduced in both directions: On the one hand, immune system components, altered by chemotherapy and donor-derived stem cells, may induce changes in the microbial community structure. On the other hand, the microbiota, altered by extensive antibiotic treatment, may influence immune parameters after HSCT. The hitherto acquired knowledge about these bi-directional relations will be the topic of this review.

MICROBIOTA DISRUPTION AFTER ALLO-HSCT AND THE ROLE OF ANTIBIOTICS

Prior to next-generation sequencing techniques that lead to increasing knowledge about the host-associated microbiotas, a standard approach in the medical treatment involved a 'gut decontamination' with the aim of limiting potential gut microbial effects in HSCT [17,18]. Under the assumption that an immunocompromised host would give way to exclusively adverse effects of potentially pathogenic anaerobic bacteria, high-dose antibiotic regimens were used, aiming at the depletion of any bacteria in the gut [18]. Eventually, controlled clinical studies were not able to document any benefit of 'gut decontamination' [19]. While prophylactic antibiotic regimens are still a standard of care for HSCT patients today, recent research indicates that the gain of preventing infections by antimicrobial prophylaxis might be compromised by adverse clinical outcomes such as GvHD and treatment-related mortality that accompany the loss of specific commensal taxa [20–24].

Common for the gut, oral, and nasal microbiota, is a decrease in bacterial alpha-diversity (hereafter referred to as 'diversity') within the first 3 weeks post-transplantation [20,25–29] (Figs 1 and 2). Loss of diversity is most pronounced in patients with GvHD [28,30–33]. Importantly, both pediatric and adult allo-HSCT patients have reduced diversity as well as an altered bacterial composition in the gut compared with healthy individuals already before the start of conditioning and transplantation [25,28]. Similarly, oral bacterial diversity has been demonstrated to be lower at the day of transplantation in adult HSCT recipients compared with

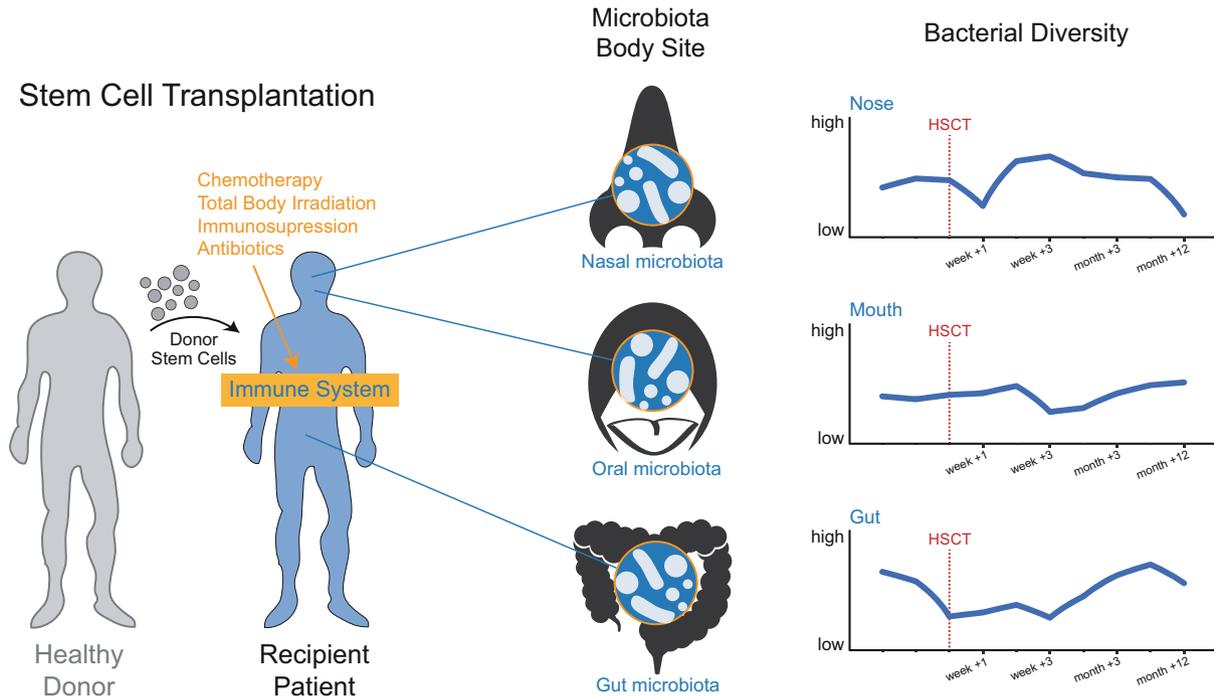


Fig. 1. General concept of allogeneic hematopoietic stem cell transplantation. The patient receives stem cells from a healthy donor. Several treatment components influence the patient’s immune system and microbiota, especially in the nasal and oral cavities, and in the gut. The bacterial diversity at mucosal sites decreases during the initial phase at slightly different time points after allogeneic hematopoietic stem cell transplantation (allo-HSCT). [Colour figure can be viewed at wileyonlinelibrary.com]

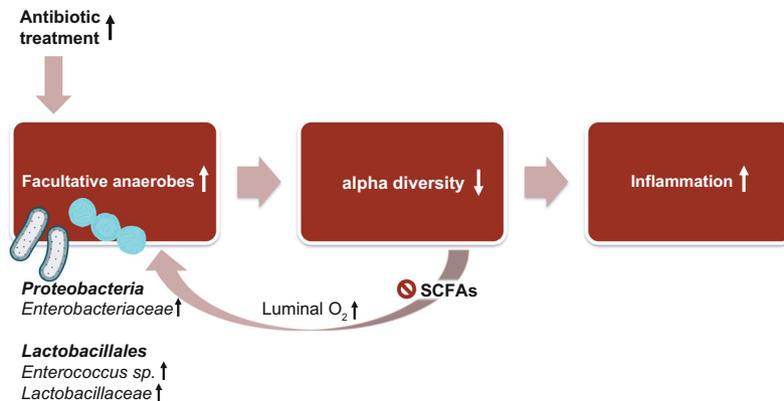


Fig. 2. Proposed detrimental mechanisms involving facultative anaerobe bacteria. Antimicrobial treatment contributes to overgrowth of facultative anaerobes, which is associated with low bacterial diversity and high inflammation. Lack of short-chain fatty acids (SCFA)-producing bacteria can reinforce growth conditions in favor of facultative anaerobes (due to high luminal oxygen). [Colour figure can be viewed at wileyonlinelibrary.com]

healthy controls [34]. This may be due to the treatment with antibiotics which causes a decrease of certain groups of commensal mucosal colonizers [21].

In addition to diversity as an approximate marker of microbial homeostasis, specific taxa have been described that increase or decrease in the gut

during the first month after allo-HSCT [20,25–27,29,31,35–37]. Microbiota members of the phylum Proteobacteria, including Enterobacteriaceae, as well as *Enterococcus* spp. and *Lactobacillus* spp., have been found to increase in abundance after transplantation [20,25,26,28,30,31,38,39] (Fig. 2). In contrast, taxa affiliated with the order Clostridiales,

in particular Lachnospiraceae and Ruminococcaceae, decreased the post-transplantation [20,25,27,40,41]. The expansion of *Enterococcus* spp. and loss of *Clostridiales* early after allo-HSCT are especially pronounced in patients with GvHD [22,31,35–37]. Furthermore, this pattern has been associated with high treatment-related mortality and blood stream infections [26,42,43]. Congruently, several genera belonging to the phylum Proteobacteria, namely *Escherichia*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, and *Stenotrophomonas*, have been found predictive for subsequent blood stream infections [44].

It has been suggested that the antibiotic treatment in allo-HSCT is promoting this shift in the gut microbial community structure [20,21,25,29,39,41,45]. Antimicrobial agents frequently used in prophylactic regimens are targeting commensal obligate anaerobe bacteria [21,41,45]. Piperacillin–tazobactam and meropenem have been described as particularly detrimental toward obligate anaerobes including Clostridiales, Negativicutes, Bacteroidetes, and Fusobacteria whereas fluoroquinolones, intravenous vancomycin, and trimethoprim-sulfamethoxazole seemed to largely spare these taxa [28,45]. A central role has been attributed to short-chain fatty acids (SCFAs), especially butyrate, produced by Clostridiales members (Fig. 3). Antibiotic-induced depletion of SCFA-producers entails a switch to glucose fermentation by enterocytes and increased oxygen availability in the intestinal lumen [46] (Fig. 2). This facilitates the expansion of facultative anaerobes such as Enterobacteriaceae (phylum Proteobacteria) and *Enterococcus* spp. [47] (Fig. 2). Within the order of Clostridiales, maintenance of high abundances of the butyrate-producers *Blautia* spp. (Lachnospiraceae) and *Faecalibacterium prausnitzii* (Ruminococcaceae) has been demonstrated to be of particular importance

for positive clinical outcomes in HSCT [48,49] (Fig. 3).

MICROBIOTA RECONSTITUTION: TEMPORAL DYNAMICS OF BACTERIAL COMMUNITY COMPOSITION

While the majority of studies have reported changes in bacterial diversity and abundances early after allo-HSCT, few studies so far have investigated long-term dynamics exceeding the first month post-transplant [25,29,37]. Recovery of intestinal diversity levels was found to first occur after 3–6 months post-transplantation in pediatric allogeneic HSCT patients [25,50] (Fig. 1). The diversity in the nasal cavity seems to recover within 2 weeks post-transplant, which is more rapid as compared with the gut and the oral cavity [25].

In a study that monitored the microbiota for up to twelve months after allo-HSCT, the bacterial composition started reconstituting to patterns similar to what was observed prior to HSCT from around three months after HSCT [25]. In this context, it is interesting to observe the recent focus on fecal microbiota transplantation (FMT) as a therapeutic approach to re-establish microbial homeostasis after allo-HSCT [51–53]. Interestingly, one study demonstrated the reconstitution of pre-transplant bacterial diversity and community structure following autologous FMT at Day +49 after allo-HSCT in adult patients [51]. However, the study also states that a small increase in gut bacterial diversity with a compromised functional repertoire of the microbiota also occurred in the control group (without FMT) at this time point [51]. Thus, while FMT might accelerate microbiota reconstitution and restore commensal functionality in allo-HSCT, intervention-free abundance trajectories should be carefully evaluated as well.

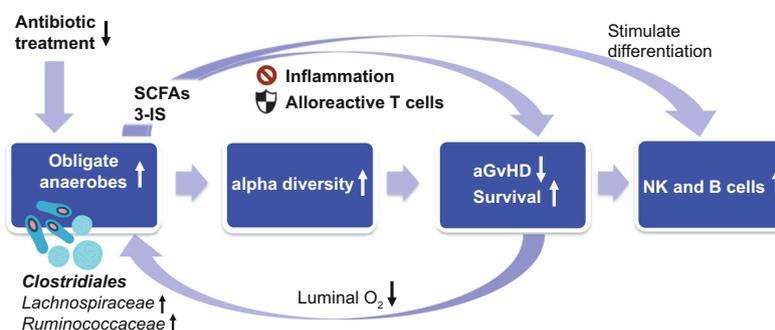


Fig. 3. Proposed beneficial mechanisms involving obligate anaerobe bacteria. Short-chain fatty acids (SCFA)-producing bacteria, such as Clostridiales, are proposed to hamper acute graft-versus-host disease (aGvHD) and promote patient survival by limiting inflammation and contributing to immune cell reconstitution after allo-HSCT. [Colour figure can be viewed at wileyonlinelibrary.com]

ASSOCIATIONS BETWEEN GUT MICROBIOTA AND HOST IMMUNE RECONSTITUTION AFTER HSCT

The reconstitution of the immune system and microbiota after allo-HSCT likely depends on each other. Abundances of specific taxa in the gut are associated with counts of distinct immune cell types after HSCT [20,25,29,54]. For instance, intestinal *Faecalibacterium* and *Ruminococcus* were found to be associated with neutrophil and monocyte reconstitution post-engraftment [54]. Moreover, NK and B-cell counts in month +1 and thereafter correlated with high counts of obligate anaerobes, including Ruminococcaceae and Lachnospiraceae [20,25] (Fig. 3). Patients with these characteristics showed milder aGvHD and higher survival [20] (Fig. 3). Consistently, these positive outcomes are associated with microbial homeostasis and high bacterial diversity, which in turn are linked to a high abundance of Ruminococcaceae and Lachnospiraceae [30,35] (Fig. 3). The production of SCFAs, in particular butyrate, by members of these bacterial families could limit inflammation and thereby prevent aGvHD to a certain extent, and this might entail a direct or indirect effect on NK and B-cell reconstitution (Fig. 3). In agreement, a study observed decreased fecal butyrate levels 14 days after allo-HSCT in patients with subsequent GvHD (grade I or higher) compared with patients without GvHD [23]. Moreover, SCFAs have been shown to facilitate the differentiation of human naive B cells to plasma cells in culture, attributing a possible direct effect of SCFAs on B-cell proliferation [55] (Fig. 3).

In line with this, a number of studies have attributed immune cell regulatory effects to butyrate, suggesting histone deacetylase inhibition as a universal mechanism [56–58]. Interestingly, histone deacetylase inhibition also has a mitigating effect on aGvHD after HSCT, in agreement with the observation of low aGvHD severity in the presence of microbial butyrate-producers [20,59]. Vice versa, the absence of aGvHD and inflammation might also benefit the retention of low oxygen levels in the gut, favoring the growth of obligate anaerobe bacteria [47] (Fig. 3).

Besides the involvement of SCFAs in shaping intestinal microbial growth conditions, direct immunomodulatory effects have been described. Butyrate is a histone deacetylase inhibitor (HDACi) and, when taken up by intestinal epithelial cells (IECs), promotes acetylation of histone H4 [60,61]. Histone acetylation generally facilitates chromatin relaxation and thereby transcription [61,62]. Reduced H4 acetylation in IECs after allogeneic HSCT has been related to decreased butyrate levels

within these cells, but not in the intestinal lumen in mice [60]. It was suggested that decreased butyrate production due to depleted butyrate-producing bacteria after allo-HSCT would not significantly affect intestinal butyrate levels because of a concurrent decrease in expression of butyrate transporter sodium-coupled monocarboxylate transporter 1 (SLC5A8) and G protein-coupled receptor 43 (GPR43), leading to reduced uptake [60]. Conversely, a human study found reduced butyrate and propionate levels in feces after allo-HSCT in pediatric patients [23]. No change in SLC5A8 and GPR43 expression was found in this study [23]. These contradicting findings warrant caution when deriving conclusions for the human HSCT setting from mouse models. Importantly, these and other studies indicate a crucial role of commensal-derived butyrate in protection from acute and chronic GvHD [23,57,60,63]. Several mechanisms have been suggested. In mice, butyrate-induced histone acetylation in IECs has been shown to upregulate expression of genes involved in junctional function and downregulate pro-apoptotic genes [60]. This suggests a direct protection against alloreactive T cells [60] (Fig. 3). Another mechanism might involve the induction of Treg cells mediated by butyrate and propionate, facilitating an anti-inflammatory milieu that protects from GvHD [57,64] (Fig. 3).

In addition to a potential protection against GvHD, propionate produced by Lachnospiraceae has also been proposed to protect against adverse effects of radiation, which has been observed in mice [65]. Mice treated with propionate exhibited elevated bone marrow cellularity and alleviated radiation-induced loss of several progenitor cell types, including granulocyte-macrophage progenitors, common myeloid progenitors, and megakaryocyte-erythroid progenitors [65]. In addition, greater colon mucus layer thickness and crypt length were observed in the propionate-treated group compared with controls, indicating a better functioning protective gut mucosal barrier [65].

In addition to SCFAs, another microbial metabolite, namely the intercellular signal molecule indole and its derivative 3-indoxyl sulfate (3-IS), has been attributed a role in allo-HSCT and GvHD [31,42,66,67]. Low 3-IS levels in urine early after allo-HSCT have been associated with low abundances of intestinal Lachnospiraceae and Ruminococcaceae and a high mortality in adult allo-HSCT patients [42]. Indole and/or its derivatives can promote tight junction integrity, hamper the growth of Gram-negative enterobacteria, and induce anti-inflammatory cytokines [67–69]. Therefore, a protective effect against GvHD has been

proposed for 3-IS [42] (Fig. 3). Consistently, low 3-IS levels are particularly pronounced in allo-HSCT patients with GvHD [31,66].

Another proposed mechanism contributing to microbial community changes after allo-HSCT involves the reduced Paneth cell numbers and accompanying decrease in human alpha defensin production during GvHD [70,71]. Paneth cell-derived AMPs contribute to gut microbial homeostasis, and the lack thereof during GvHD facilitates overgrowth and domination of opportunistic pathogens, such as *Escherichia coli* in mice [70,71]. Consistently, a human study showed that adult allo-HSCT patients with severe gastrointestinal GvHD exhibited low Paneth cell numbers, low Paneth cell-derived AMP expression, and low urinary 3-IS, the latter pointing to low bacterial diversity [72].

BEYOND THE GUT—THE ROLE OF THE ORAL AND NASAL MICROBIOTA IN ALLOGENEIC HSCT

The gut is clearly the microbial niche most studied in allo-HSCT patients, but other body sites, such as the oral cavity, have also been examined in a few studies [25,29,34,73]. For instance, in adult patients, the presence of *Staphylococcus haemolyticus* and *Ralstonia pickettii* in the oral cavity on the day of transplantation was associated with a higher mortality risk [34]. Lesions of oral mucositis, which is a common oral complication in patients undergoing allo-HSCT, have been found to be colonized with *Fusobacterium nucleatum* [74]. Oral microbiota profiles of pediatric patients could be discriminated at different time points prior to and post-allo-HSCT by the abundances of distinct Actinomycetaceae, Streptococcaceae, and Prevotellaceae [25]. For example, distinct Prevotellaceae exhibited reduced abundances after allo-HSCT in pediatric patients, as compared with before allo-HSCT [25]. *Prevotella* spp. have also been described to decrease in adult patients after allo-HSCT [73]. High pre-transplant abundances of a certain oral *Prevotella melaninogenica* taxon predicted subsequent moderate to severe aGvHD [25]. *Prevotella* might play a universal role in regulating inflammation in allo-HSCT. However, 16S rRNA gene profiling might not provide a taxonomic resolution high enough to determine which particular members of the Prevotellaceae family are responsible for pro-inflammatory signaling and which might point to microbial homeostasis, but suggest ambiguous influences potentially depending on the particular species, if not the strain.

Interestingly, host-microbial associations of the oral and nasal microbiota reflect host-gut microbial associations to a large extent [25]. Associations were shared between body sites in the sense that the same sets of specific immune cell types or immune markers exhibited correlations with the microbiota at two or three body sites. For instance, CD4+ T cell and TH17 cell counts in pediatric allogeneic HSCT patients were associated with a group of Ruminococcaceae and Lachnospiraceae in the gut, as well as with a group of Veillonellaceae in the nasal cavity, and a diverse group of taxa including Flavobacteriaceae in the oral cavity [25]. This could indicate a pro-inflammatory immunomodulatory involvement of the microbiota at multiple body sites. This might serve as an example for microbiota in different niches and their shared correlations with immune markers in HSCT. However, in some cases, these associations were strongly dependent on the patients' clinical baseline parameters [25].

PREDICTION OF AGVHD FROM PRE-TRANSPLANT *LACTOBACILLUS* SP. AND OTHER GUT, ORAL, AND NASAL MICROBIAL TAXA

A number of studies have related the pre-transplant microbial community structure to clinical outcomes after HSCT [20,25,29,33,37,75,76]. For instance, an increase in Lactobacillaceae prior to aGvHD onset was observed in patients who were still alive after 5 years on average [20]. High abundances of a specific *Lactobacillus* sp. taxon before allo-HSCT and up to the time of transplantation predicted the development of moderate to severe aGvHD [25]. Lactobacillaceae expansion might be a part of a protective effect in reaction to microbial community disruption and inflammation. The concurrent high levels of the antimicrobial peptide human beta defensin 2 (hBD2) might point to both, a high burden of inflammation and a high number of opportunistic pathogen infections in the patients in question [20]. It has been suggested that specific probiotic *Lactobacillus* spp. can induce the secretion of hBD2 in immune cells [77,78]. Microbial community disruption after allo-HSCT has been associated with an increase in Enterococcaceae in a number of studies [17,31,35]. It has been suggested that a concurrent increase in Lactobacillaceae limits Enterococcaceae expansion [35]. This could be interpreted as a protective reaction to facilitate the re-establishment of microbial homeostasis. Consistently, temporal trajectories of certain closely phylogenetically related Lactobacillaceae taxa have

been identified, showing an increase immediately after HSCT that reflects the trajectory of Enterococcaceae [25]. In summary, the immunomodulatory characteristics attributed to Lactobacillaceae and its abundance patterns suggest this family as a potential marker of a state of inflammation and loss of microbial homeostasis prior to and at the time of allo-HSCT, which might influence the development of aGvHD (Fig. 2).

Additional gut taxa have been identified that might have a predictive value in aGvHD, including a distinct *Parabacteroides* spp. taxon and a taxon affiliated with the Lachnospiraceae family [25]. Furthermore, it has been demonstrated that pre-transplant abundances of oral and nasal microbiota members could predict subsequent aGvHD [25].

With regard to mortality, high abundances of Firmicutes, *Enterococcus*, Bacilli, as well as *Streptococcus* sp. DN812 and *Veillonella parvula* one to three weeks after transplantation were predictive for increased mortality after HSCT [28]. In contrast, high abundance of Clostridia, Streptococcaceae, and Lactobacillaceae predicted reduced mortality during this period [28].

CONCLUSION

A crucial role for mucosal microbiotas in stem cell transplantation is supported by a significant body of research. Disturbance and reconstitution of the microbiota and the immune system follow concurrent timelines and depend on each other. This holds true for the oral and nasal microbiota, as well as for the gut microbiota. To retain bacteria associated with rapid immune system recovery, cost and benefit of antimicrobial treatment prior to and after HSCT have to be carefully weighed, and commensal-sparing antimicrobial agents should be favored. Importantly, clinical outcomes after allo-HSCT, such as GvHD and mortality, are predictable from pre-transplant bacterial abundances. This knowledge can aid the development of personalized treatment strategies, such as intensified prophylactic immunosuppression for patients at increased risk for GvHD. Future studies will have to assess the microbiota in relation to the metabolome of mucosal sites as well as other factors in allo-HSCT patients to facilitate an understanding of the underlying mechanisms.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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