Blood Reviews xxx (xxxx) xxx

Contents lists available at ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/issn/0268960X

Review

Targeting the gut microbiome: An emerging trend in hematopoietic stem cell transplantation

Sona Ciernikova [a,*], Barbora Kasperova [b,c], Lubos Drgona [b,c], Bozena Smolkova [d], Viola Stevurkova [a], Michal Mego [c,e]

[a] Department of Genetics, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia
[b] Department of Oncohematology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
[c] National Cancer Institute, Bratislava, Slovakia
[d] Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia
[e] 2nd Department of Oncology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

ARTICLE INFO

Keywords:
Microbiome
Bacterial diversity
Cancer treatment
Hematopoietic stem cell transplantation
Graft-versus-host disease
Fecal microbiota transplantation
Probiotics

ABSTRACT

Mounting evidence has demonstrated the critical role of the gut microbiome in different cancer treatment modalities showing intensive crosstalk between microbiota and the host immune system. In cancer patients receiving hematopoietic stem cell transplantation (HSCT), conditioning regimens including chemotherapy, radiotherapy, and immunosuppressive therapy, as well as antimicrobial prophylaxis, result in intestinal barrier disruption and massive changes in microbiota composition. According to clinical studies, a drastic loss of microbial diversity during HSCT is associated with enhanced pro-inflammatory immune response and an increased risk of transplant-related complications such as graft-versus-host disease (GvHD) and mortality. In this review, we outline the current understanding of the role of microbiota diversity in the patient response to cancer therapies and highlight the impact of changes in the gut microbiome on clinical outcomes in post-HSCT patients. Moreover, the therapeutic implications of microbiota modulation by probiotics, prebiotics, and fecal microbiota transplantation (FMT) in hematologic cancer patients receiving HSCT are discussed.

1. Introduction

Host physiology may be affected by a dynamic balance between eubiosis and dysbiosis of intestinal microbiota. The microbial community and host factors together influence nutritional biotransformation, immune response, and xenobiotic metabolism [1]. Joshua Lederberg firstly designated the microbiome as "the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease" [2]. In current understanding, the human microbiome comprises all the genetic material within the microbiota that resides in the human body. Predominantly, there are three methods of obtaining microbiome data: i) 16S rRNA gene sequencing defining microbiome diversity, ii) metagenomic analysis used to portray functional potential, and iii) metatranscriptomic approach assessing active gene expression [3]. Progress in comprehensive multi-omics technologies, together with the development of sophisticated bioinformatics algorithms have allowed extensive microbial analyses to identify even uncultivated microorganisms, and brought us closer to understanding the true influence of the microbiome on human health [4–6].

The Human Microbiome Project (HMP) Consortium, as well as the MetaHIT Consortium, found that the microbiome diversity with strong niche specialization varies within a single subject and among healthy subjects [7,8]. Enterotypes, body mass index, as well as lifestyle, dietary and cultural conventions, are among the main factors explaining the inter-individual differences [9]. However, specific tissue sites are associated with the preservation of an established eco-system to exert distinct functions. Due to its enormous impact on the host's homeostasis, the gut microbiota is the most extensively studied human microbial community [10]. As reported previously, Bacteroidetes and the Firmicutes are two major predominant phyla constituting over 90% of the known phylogenetic categories [11]. Combining fecal metagenomes of subjects from European countries with previously published datasets of Japanese and American individuals, three robust enterotypes that are

* Corresponding author at: Department of Genetics, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovakia.
E-mail address: sona.ciernikova@savba.sk (S. Ciernikova).

https://doi.org/10.1016/j.blre.2020.100790

Available online 26 December 2020

not a nation- or continent-specific were identified. According to these results, "the dominant gut microbial phyla are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobiat. The Firmicutes phylum is composed of more than 200 different genera such as *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminicoccus*. *Clostridium* genera represent 95% of the Firmicutes phyla. Bacteroidetes consist of predominant genera such as *Bacteroides* and *Prevotella*. The Actinobacteria phylum is proportionally less abundant and mainly represented by the *Bifidobacterium* genus" [12]. Geography has been identified as one of the main factors contributing to large-scale microbiome alterations. International studies comparing the microbial data between North and South America [13], Europe and Africa [14], Korea and Japan [15], rural vs. urban populations of Russia [16] and China [17] showed systematic differences in healthy human microbial composition among studied populations. Accordingly, by comparison of the three largest cohorts— MetaHIT (European), HMP (American), and a Chinese diabetes gene catalogs—the variation in taxonomic composition was revealed [18].

A large body of evidence has linked the human gut microbiota to cancer, so targeting the microbiome in cancer treatment faces mounting research interest. Cancer patients undergoing allogeneic hematopoietic stem-cell transplantation (HSCT) are characterized by an elevated risk of immune-related complications resulting from the nature of the disease and undergoing therapy. Due to its interaction with the host immune system, intestinal microbiota and its considerable effect on the clinical outcome of post-HSCT patients have been intensively studied. To eradicate cancer cells and induce immunosuppression, a preparative conditioning regimen including high-dose chemotherapy/radiation therapy is given to HSCT patients. However, both treatments result in massive microbiota reconstruction, and disruption of the homeostatic dialog between the gut microbiota and the host immune system leads to dysbiosis followed by serious complications [19]. Here we provide the current knowledge concerning the role of the microbiome in cancer treatment with the main focus on the most recent data related to HSCT preceded by extensive conditioning regimens. Importantly, the impact of microbiome modifications on clinical outcomes of post-HSCT patients will be discussed. A deep understanding of HSCT-related microbial changes and their associations with severe post-transplant complications might bring new perspectives for microbiota-mediated interventions.

2. Hematopoietic stem cell transplantation

HSCT has gone through great development since the 1990s and thanks to its curative potential, it became a routine therapeutic option for a wide variety of malignant and non-malignant diseases. Bone marrow, peripheral blood stem cells, and umbilical cord blood are all potential sources of hematopoietic stem cells. HSCT allows the use of high-dose cytotoxic drugs sometimes in combination with radiotherapy, which provides sufficient immunoablation, significant antitumor effect, and clearance of "space" in the bone marrow. Severe hematological toxicity is eliminated by consecutive administration of stem cells, helping to restore hematopoiesis. Chemotherapy regimens vary according to the type of transplantation, autologous (auto-) vs. allogeneic (allo-) HSCT, or primary disease. Moreover, a combination of regimens is often used, except solo melphalan in multiple myeloma, with alkylating agents, platinum analogs, topoisomerase inhibitors, adenosine deaminase inhibitors, and pyrimidine antagonist cytarabine among the most frequent drugs used. Conditioning regimens have been classified as high-dose- myeloablative, reduced-intensity, and/or nonmyeloablative [20]. In allogeneic-HSCT (allo-HSCT), the immune response of donor cells against host cancer cells - the so-called graft-versus-tumor (GvT) effect - enhance the antitumor activity. Reduced-intensity and non-myeloablative conditioning regimens have therefore come to the foreground in order to reduce acute and late toxicity and thus make HSCT available to older and frail patients [21]. HSCT recipients are at substantial risk for a variety of acute and late complications that impair quality of life and increase mortality, including recurrence of the underlying disease, infections, second cancers, and organ system dysfunction. The complications are more severe for those who have received allogeneic transplants, especially imminent graft-versus-host disease (GvHD), occurring when the donor's white blood cells attack the healthy recipient's tissues. GvHD is thought to be primarily initiated by hematopoietic dendritic cells. Interestingly, animal studies have shown recipient non-hematopoietic antigen-presenting cells (APCs) are able to induce CD4+ T cell-dependent acute GvHD as well [22]. Life expectancy after HSCT differs from that of the general population. The mortality rate among HSCT patients remains elevated for decades after treatment, approaching that of the general population over time [23–25].

In the last decades, the outcome of patients undergoing auto- or allo-HSCT has been improved due to better supportive care, improvements in conditioning regimens, and a better understanding the mechanism of toxicity and restoration of hematopoiesis. At the same time, due to these advances, there is an increase in the number of HSCT with a higher risk of toxicity, like HSCT in older patients, or allogeneic stem cell transplantation from unrelated donors. This trend further emphasizes the need for further comprehension of the underlying pathophysiology and the role that the gut microbiome plays in these processes.

3. The gut microbiome and cancer

The growing evidence support the association between disruptive changes in the gut microbiome and tumor development. The study of microbiome alterations during tumorigenesis in a mouse model of the inflammation-driven colon cancer revealed that the microbiome profile in the tumor-bearing and non-tumor-bearing animals differ to a large extent. Moreover, these changes were directly responsible for tumorigenesis as introducing gut microbiota from tumor-bearing mice to germ-free (GF) ones resulted in significantly more frequent tumor development [26].

Several factors influence the microbiome in cancer patients leading to the microbial changes that might affect different aspects of patient outcomes (Fig. 1). Preclinical in vitro research and animal models, together with the clinical studies, have shown the microbiome is extensively modified by cancer treatments such as chemotherapy, radiotherapy, and immunotherapy. At the same time, it has been observed that the individual composition of bacterial microbiota can potentiate the immune system and acts synergistically with cancer treatment [27].

3.1. Cancer treatment modifies the gut microbiome

Treatment with DNA-alkylating agent cyclophosphamide (CY) disrupted gut barrier integrity and perturbed intestinal homeostasis, leading to host immunization against some bacterial strains [28]. Viaud et al. showed CY-induced changes in the microbial composition of the small intestine and significant translocation of selected Gram-positive bacteria (*Lactobacillus johnsonii*, *Lactobacillus murinus,* and *Enterococcus hirae*) into mesenteric lymph nodes and spleen in sarcoma mouse models. Moreover, CY treatment increased the frequency of "pathogenic" Th17 (pTh17) cells expressing hallmarks of both Th1and Th17 cells within the spleen. The gut microbiota-dependent manner was confirmed by treatment with antibiotics towards Gram-positive bacteria, while GF tumor-bearing mice were resistant to CY and reported reduced levels of pTh17 cells [29].

The resistance of GF mice to radiation-induced enteritis, as well as post-radiotherapy changes in gut microbial diversity, point out to the role of gut microbiota in regulating the response and repair of irradiation-induced damage [30,31]. The results of a study among irradiated and control mice demonstrated dysbiosis after rectal radiation, showing enhanced IL-1β and TNFα expression in post-radiation

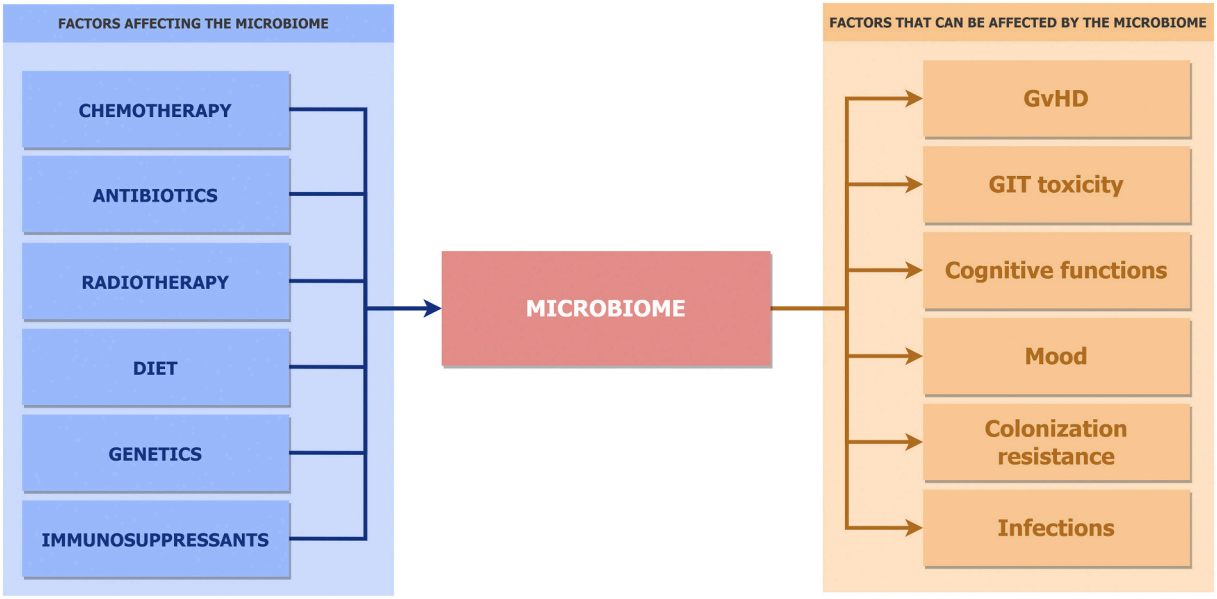


Fig. 1. The emerging role of the microbiome in cancer patients. A schematic diagram summarizing the main factors affecting the patients' microbiome (left panels), as well as the factors which might be affected by microbiome changes (right panels) in cancer patients treated with currently available anticancer therapy.

microbiota. GF mice colonized by irradiated microbiota were predisposed to radiation injury and colitis. According to the authors, "microbial-induced radiation tissue damage was at least in part mediated by IL-1β, considering microbiota manipulation or direct inhibition of IL-1 may represent a potential therapeutic approach for reducing intestinal radiation-induced mucosal toxicity" [32]. The radiation-induced shift in gut microbiota composition has been detected not only in mouse models but also in human pathologies. A prospective study on gynecological cancer patients receiving pelvic radiotherapy showed significant gut microbiota alterations and reduction in species-level taxa in cancer patients comparing to healthy subjects. According to the findings, radiation therapy decreased Firmicutes phyla by 10%. In contrast, the phyla Fusobacterium was increased by 3% [33]. In accordance, the use of 16S rRNA gene sequencing confirmed a decrease in diversity and richness of gut microbiota in a cohort of 11 patients with different cancer types five-week post-pelvic radiotherapy. Importantly, the study results suggested using pre-existing changes in Firmicutes/Bacteroidetes ratio as a predictive tool for developing post-treatment diarrhea [34]. Recently, remarkable changes in gut microbiota profiles following radiation enteritis (RE) were reported on fecal samples from 18 cervical cancer patients showing a significant reduction of α-diversity, while β-diversity was increased. The patients with RE were characterized by a relatively higher abundance of Proteobacteria and Gammaproteobacteria, and a lower abundance of *Bacteroides* spp. Additionally, RE patient-derived bacterial-epithelial co-cultures confirmed enhanced TNF-α and IL-1β expression previously reported in mice studies [35]. The largest clinical study recruited 134 patients treated by radiotherapy without cytotoxic systemic therapies, presented an association of low bacterial diversity and higher counts of *Clostridium IV*, *Roseburia*, and *Phascolarctobacterium* with delayed radiation-induced enteropathy [36].

Taken together, these data suggest that anticancer and supportive treatments modify the microbiome composition in cancer patients, and vice versa, microbiome modifies treatment effectivity and toxicity. A better understanding of this relationship and its subsequent therapeutic modification is important to improve the outcome of cancer patients.

3.2. Microbiome modifies the response to cancer treatment

The impact of gut microbiota on chemotherapy effects was described by the "TIMER mechanistic framework describing Translocation, Immunomodulation, Metabolism, Enzymatic degradation, and Reduced diversity and ecological variation" [37]. The breakthrough findings coming from animal studies showed that disruption of the microbiota in antibiotic-treated or GF mice impaired the response of subcutaneous tumors to platinum chemotherapy. These studies showed that GF mice did not respond to oxaliplatin drug treatment due to the decreased cytotoxicity and deficiency in reactive oxygen species (ROS) production. However, chemotherapeutic efficiency was restored after the administration of lipopolysaccharide. As the authors concluded, particular members of intestinal microbiota might promote increased oxidative stress and tumor cell death by the production of toll-like receptor (TLR) agonists [38]. Accordingly, bigger tumors and reduced survival were observed in mice treated with cisplatin coupled with antibiotics comparing to the animals treated with cisplatin alone. Gene expression study indicated that antibiotics-induced upregulation of VEGF-A expression and downregulation of BAX and CDKN1B expression can partially diminish the efficacy of cisplatin. Moreover, antibiotic treatment reduced the expression of IFN-γ, GZMB, and PRF1 in the CD8(+) T cells of treated mice. On the other hand, the combination of cisplatin with probiotics showed an increased therapeutic response, smaller tumors, and an improved survival rate due to the induction of pro-apoptotic genes and enhanced host immune response [39].

Cancer immunotherapy represents a therapeutic modality successfully used in the treatment of hematological and solid metastatic cancers [40–42]. However, studies on GF mice as well as in mice treated with broad-spectrum antibiotics show reduced therapeutic effects of immunotherapy due to a combination of TLR9 antagonist and antibody to interleukin-10R [38], or administration of antibodies against CTLA-4 [43] and/or receptor/ligand system programmed death-1 (PD-1/PD-L1) [44]. As Iida et al. reported, the intact gut microbiota has mediated thetherapeutic effects of CpG-oligonucleotide immunotherapy by modulating myeloid-derived cell functions in the tumor microenvironment [38]. Vétizou et al. revealed immunotherapy directed against CTLA-4, a major negative regulator of T cell activation, was not efficient in the treatment of tumors in antibiotic-treated or GF animals. The antitumor therapeutic efficacy of the CTLA-4 blockade was shown to be dependent on distinct *Bacteroides* spp., particularly *B. thetaiotaomicron* or *B. fragilis* via activation of IL-12-producing dendritic cell and T-cell helper (Th1) responses. Moreover, microbial fecal transplantation of *Bacteroides* spp.-rich feces from humans into GF mice induced a

significant response to CTLA-4 blockade, and negatively correlated with tumor size in recipient mice [43]. Interestingly, the study on melanoma-bearing mouse models revealed the role of *Bifidobacterium* spp. in enhancing anti-tumor immunity and raising the efficacy of PD-L1 blocking therapy. According to the findings, cohousing or fecal transfer eliminated the differences in spontaneous antitumor immunity between mice harboring distinct commensal microbiota [44]. Very recently, Mager et al. have showed a link between the response to cancer

Table 1
Modifications of gut microbiome during Hematopoetic Stem Cell Transplantation (HSCT).

Study	Study design	Disease	Purpose	Patients (n)	Intervention	Study status
NCT03529825	A pilot, single-arm prospective study with a retrospective control arm	Hematologic malignancies, allo-HSCT	To see if rifaximin can help to balance gut bacteria and improve transplant outcomes, reduce the risk of infection	66 (children only)	Experimental arm: Rifaximin orally 15 mg/kg divided BID during HSCT	Recruiting
NCT04111471	An interventional randomized prospective study, two cohorts: Inulin/ placebo	Hematologic malignancies, allo-HSCT	Compare the effect of oral inulin vs. placebo on bacterial diversity in the stool and short-chain fatty acids levels	40 (children only)	Prebiotics cohort will receive 10 g of inulin product daily for a total of 21 days during HSCT	Recruiting
NCT04281797	An observational prospective pilot study	Recipients of kidney transplantation, liver transplantation, allo-HSCT, and mesenchymal SCT	To investigate the alterations of the gut microbiome throughout transplantation in association with the clinical outcomes	90	/	Enrolling by invitation
NCT01371656	An interventional randomized controlled phase 3 study	Acute leukemia patients and HSCT recipients receiving intensive chemotherapy	Comparison of the incidence of bacteremia between levofloxacin vs. no prophylaxis arm	624	Levofloxacin receiving orally or intravenously after chemotherapy administration 2 consecutive courses or during HSCT	Completed
Study results: "Usage of levofloxacin resulted in a significant reduction in bacteremia among children with acute leukemia receiving intensive chemotherapy (21.9% vs 43.4%; risk difference, 21.6%; 95% CI, 8.8%–34.4%, $P = 0.001$). There was no significant reduction in bacteremia among children undergoing HSCT." [55]						
NCT02966457	An interventional randomized phase 4 study	Hematologic malignancies and recipients of HSCT with ESBL-producing or carbapenem-resistant bacteria	To evaluate the efficacy and safety of selective MDR gram-negative bacteria decolonization	62	Intestinal decolonization with colistin (2 mln I.U. 4×/day orally) for 14 days in the experimental arm	Completed
Study results: "The incidence of bloodstream infections was lower in the first 30 days after the intervention (3.2% vs. 12.9%), but overall, it did not show any advantages within the 90-day observation period." [56]						
NCT03557749	An observational prospective study	Recipients of HSCT and their donors, patients treated with novel immunotherapy	Monitoring of immune and microbial reconstitution in HSCT and novel immunotherapies	1600	/	Recruiting
NCT00398411	A pilot, double-blind, randomized, placebo-controlled study	Lymphoma, multiple myeloma or solid tumor patients undergoing auto-HSCT	To assess the efficacy and safety of moxifloxacin in the prevention of bacteremia after HSCT, its effect on overall survival and antibiotic sensitivity of blood isolates	66	Moxifloxacin 400 mg orally once daily during auto-HSCT in the experimental arm	Completed
Study results: "Moxifloxacin prevented bacteremia (8.8% vs 28.1%, $P = 0.042$) and shortened febrile episodes (9.5 days vs. 7.69 days, $P = 0.0499$) in patients receiving auto-HSCT. No significant increase in adverse events in the moxifloxacin arm was observed, possibly due to the rather small sample size." [57]						
NCT03922035	An interventional randomized open label pilot study	Recipients of allo-HSCT	To study the efficacy, side effects, and impact of CBM588 on improving the clinical outcomes, increase in bacterial biodiversity, and gastrointestinal toxicity prevention	36	*Clostridium butyricum* CBM 588 probiotic strain orally BID from a day of admission to day 28 (with the absence of disease progression or unacceptable toxicity)	Recruiting
NCT03078010	An interventional randomized open-label phase 2 study	Recipients of allo-HSCT	To investigate the effect of antibiotics on inflammatory reactions and the community of intestinal *Clostridiales* spp.	144	Cefepime (2 g IV q 8 h) used for the treatment of febrile neutropenia in the experimental arm	Recruiting
NCT02763033	An interventional randomized phase 2 study	Recipients of allo-HSCT	To evaluate the feasibility and efficacy of a dietary supplement containing potato-based resistant starch to increase the intestinal levels of butyrate and reduce aGVHD	70	In the experimental arm, the resistant potato-based starch will be added to the standard HSCT diet compared to placebo non-resistant starch	Recruiting
NCT02641236	An interventional randomized phase 2 study	Pediatric recipients of allo-HSCT	To study post-HSCT effects of gut decontamination with vancomycin-polymyxin B and the impact on aGVHD incidence	28 (children only)	Participants in the experimental arm will receive non-absorbable, oral vancomycin-polymyxin B	Recruiting
NCT03942159	An observational prospective study	Recipients of allo-HSCT and donors	To investigate the microbiome composition in both recipients and donors, and reveal the changes in post-HSCT biodiversity	15	/	Recruiting

Abbreviations: ATB, antibiotics; BID, twice a day; ESBL–producing bacteria, extended spectrum beta-lactamase–producing bacteria; HSCT, hematopoietic stem cell transplantation; MDR, multidrug resistance; SCT, stem cell transplantation.
The table summarizes the list of ongoing and completed clinical trials evaluating the effect of novel immunotherapy, gut microbiota modifications by antibiotics, probiotics or prebiotics on bacterial diversity, prevention of bacteremia, improvement of clinical outcomes, and overall survival in post-HSCT patients (according to https://clinicaltrials.gov/).

immunotherapy in mice and microbiota-derived metabolite inosine [45].

Animal models highlight the importance of the microbiome in immune checkpoint blockade (ICB) immunotherapy and contribute to the growing consensus of the existing link between gut microbiota and ICB immunotherapy efficacy in cancer patients. Results from a study that included 249 patients with metastatic melanoma, non-small cell lung cancer, and renal cell carcinoma on ICB treatment demonstrated that antibiotic usage inhibited the clinical benefit of immune checkpoint inhibitors (ICIs) targeting the coinhibitory PD-1/PD-L1 [46]. The differences in systemic and anti-tumor immune responses depending on the gut microbiome status were also detected among 112 melanoma patients treated with PD-1 blockade showing enhanced responses in patients with high diversity and abundance of Ruminococcaceae/*Faecalibacterium* genus [47].

These data suggest, that the microbiome composition modulates the response to cancer treatment and this is especially true for currently used immunotherapeutic strategies. With a high probability, a single change in microbiome composition is not responsible for these conditions, but this interaction will be more complex. Further research should focus on patients and microbiome-related factors that modulate therapeutic response, including interactions between microbiome, host immune system, intestine, food composition as well as the influence of anticancer and supportive therapy.

4. The microbiome and HSCT

Considering the important role of the microbiome and its modulation in cancer patients treated with chemotherapy, radiation therapy, and immunotherapy, the connection between microbiota and HSCT has been evaluated. Mucosal barrier disruption due to extensive conditioning regimens allows translocation of bacteria to the intestinal mucosa, inducing excessive immune responses via inflammatory cytokines, which result in intestinal inflammation. Moreover, damaged integrity of the intestinal epithelial barrier allows microbes to enter the blood circulation resulting in bloodstream infections (BSI) and sepsis. Enterobacteriaceae and staphylococci followed by enterococci, *Pseudomonas aeruginosa*, and viridans streptococci represent the most common BSI pathogens [48]. BSI has been shown to occur in 20–60% of patients receiving high-dose chemotherapy, with sepsis-associated mortality ranging from 9% to 31% [49–51]. A large number of studies have demonstrated an association between antibiotic prophylaxis and the risk of antibiotic-resistant bacterial infections. Interestingly, metagenomic analysis of the human gut and saliva microbiota discovered previously unknown resistance genes [52]. Broad-spectrum antibiotic prophylaxis reduced the number of transmigrated bacteria in HSCT patients during the early phase, but long-term administration caused remarkable shifts and losses of microbial diversity leading to unfavorable effects. Moreover, individual variations in resembling pre-treatment microbiota community after the treatment have been observed [53,54]. Numerous clinical trials evaluating the effect of gut microbiome modifications on the prevention of bacteremia and improved clinical outcome of HSCT are currently ongoing (Table 1), some of them have been already completed [55–57].

As shown earlier, anticancer and supportive treatment actively modified the microbiome composition. However, these changes are more pronounced in the setting of HSCT with the direct implication for the effectivity and toxicity of this approach. Therefore, the processes of microbiome modification become the emerging field of HSCT research.

4.1. Allogeneic HSCT

Acute GvHD (aGvHD) is a major cause of non-relapse mortality in patients after allo-HSCT [58]. According to the findings, aGVHD is linked to the disruption of intestinal microbiota diversity, showing the higher mortality and aGvHD incidence in patients receiving prophylactic antibiotic treatment [59–61]. The use of microbial biomarkers and targeted antibiotics, respecting the results from patients' microbiome analysis, might be an option. Studies assessing the variations in pre- and post-transplant composition of gut microbiota reported an extremely reduced bacterial diversity after treatment, associated with an increased risk of GvHD and mortality (Table 2).

According to Taur et al., multivariate adjustment for clinical predictors detected a strong effect of low microbial diversity on mortality in allo-HSCT recipients. Interestingly, lower diversity was characterized by the dominance of single bacterial genus mostly of genera *Enterococcus*, *Streptococcus*, Enterobacteriaceae (*Escherichia* and *Kluyvera*), and *Lactobacillus* [62]. Pre-HSCT microbial profiling of patients receiving allo-HSCT and the healthy donors showed lower fecal bacterial diversity and different phylogenetic membership in HSCT recipients. Importantly, the association between high bacterial donor diversity and decreased risk of aGvHD was evaluated [63]. In the study monitoring the microbiome of pediatric cancer patients during allo-HSCT, some differences were observed in the diversity indices in affected children compared with those not affected by GvHD [64]. A clinical study of 107 allo-HSCT recipients analyzing fecal microbiota composition 2 weeks prior conditioning regimen reported significant differences in abundance of phylum Firmicutes and Bacteroidetes between aGVHD and non-aGvHD patients. As the authors concluded, a possible preventive strategy against aGvHD might be the maintenance of Bacteroidetes throughout the treatment [65]. Furthermore, a large retrospective single-center study of stool samples from 541 allo-HSCT patients showed an association between a higher abundance of *Eubacterium limosum* and a decreased risk of disease relapse/progression [66]. However, some findings indicated that reduced GvHD-related mortality and improved overall survival in allo-HSCT patients might be associated with the presence of *Blautia* spp. [67]. In the latest multi-center international study, Peled et al. observed the association between higher microbial diversity and lower risk of mortality in an extensive cohort of allo-HSCT patients. Importantly, the geographic location of patients did not change the pattern of microbial disruption [68].

Gut microbiota and its metabolites promote intestinal tissue homeostasis and promote immune tolerance to infections post-allo-HSCT [69]. A relative shift towards enterococci in post-transplant stool specimens has been observed, resulting in a mean enterococci proportion corresponding to 21% within non-GvHD patients compared to 46% in patients developing GvHD. Moreover, the mean proportion raised to 74% at the time of aGvHD, while *Enterococcus faecium* was the most significantly increased bacterium [70]. Similarly, a multicenter international study on a large cohort consisting of more than 1300 patients receiving allo-HSCT detected the high fecal appearance of enterococci in the early post-HSCT period. The results confirmed and extended previous findings of the correlation between *Enterococcus* spp. domination and GVHD-related mortality and worse outcome of HSCT patients, suggesting a potential therapeutical implication of this observation [71]. Very recently, findings of Kusakabe et al. have encouraged the detection of increased enterococci relative abundance as a prognostic predictor of poor prognosis in patients receiving allo-HSCT. The results showed worse overall survival for those allo-HSCT patients with Enterococcus relative abundance equal to or higher than 1% [72].

The predominance of enterococcal strains may diminish various *Clostridiales* spp., the most abundant non-pathogenic commensal gut bacteria, and thus lead to an increased incidence of GvHD. The majority of *Clostridiales* were considered to play an important role in maintaining tolerance against the intestinal microbiome by inducing regulatory T cells via butyrate production [73,74]. Butyrate, a bacterial metabolite of anaerobic fermentation, serves as an important substrate for intestinal epithelial cells [75] and influences enteric immune tolerance against pathogens [76]. Moreover, butyrate is associated with increased histone acetylation followed by raised expression of anti-apoptotic proteins JAM, and occludins participating in barrier integrity and tight junction assembly [77]. In accordance, the study on mouse models reported

Table 2
Microbiome changes in patients receiving hematopoetic stem cell transplantation (HSCT) and the impact on GvHD and/or clinical outcome.

Purpose	Study design	Patients (n)	Major findings	Study [Ref.]
To investigate the epidemiology and clinical impact of gut colonization resistance on OS of HSCT-patients	A single-center retrospective study	107 patients undergoing allo-HSCT	Gut colonization resistance decreased OS of allo-HSCT patients by increasing nonrelapse mortality and the incidence of systemic infection and aGVHD	Bilinski et al. [59]
To evaluate the associations between antibiotics use to treat neutropenic fever and GvHD-related mortality	A single-center retrospective study	857 recipients undergoing allo-HSCT	Selecting antibiotics to treat neutropenic fever could prevent dysbiosis and reduce mortality associated with GvHD in allo-HSCT patients	Shono et al. [61]
To assess the impact of MDRO colonization on overall survival of auto-HSCT patients	A single-center retrospective study	184 patients receiving auto-HSCT	Outpatient care of auto-HSCT patients with MDRO colonization is highly recommended after discharge from the hospital	Scheich et al. [81]
To find out whether microbiota changes in auto-HSCT patients would reflect dysbiosis seen in allo-HSCT patients	Two centers retrospective study	365 patients undergoing auto-HSCT	Auto-HSCT patients showed significantly lower pre-transplant diversity than healthy controls. Loss of diversity and dysbiosis was comparable after auto- and allo-HSCT, but auto-HSCT patients exhibited a more rapid recovery	Khan et al. [82]
To determine the impact of intestinal biodiversity on post-transplant mortality outcomes	A prospective study, 16S rRNA sequencing of fecal samples	80 recipients undergoing allo-HSCT	The intestinal microbiota might be an important factor in the success or failure of allo-HSCT. Low diversity was strongly associated with the mortality	Taur et al. [62]
To elucidate the variations in the gut microbiome among HSCT patients	A prospective study, 16S rRNA sequencing of fecal samples	31 patients receiving allo-HSCT	A relative shift towards Enterococcal domination in patients with aGvHD	Holler et al. [70]
To evaluate the role of gut bacteria in GvHD pathophysiology	A prospective study, 16S rRNA sequencing of fecal samples	64 patients undergoing allo-HSCT	An association between increased bacterial diversity and reduced GvHD-related mortality. A link between genus *Blautia* and reduced GvHD lethality and improved OS was observed	Jenq et al. [67]
To analyze the gut microbiome of patients undergoing allo-HSCT and describe the microbial changes	A prospective study, metagenomic and metatranscriptomic analysis of fecal samples	16 patients undergoing allo-HSCT	Reduction of bacterial diversity in post-allo-HSCT patients and adverse effect of prophylactic antibiotic administration on the treatment outcomes	Kaysen et al. [60]
To study the associations between aGvHD and bacterial profiles before preparative conditioning in patients and their paired HLA-matched sibling donors	A prospective study, 16S rRNA sequencing of fecal samples	57 patients receiving allo-HSCT 22 paired HLA-matched sibling donors	Different phylogenetic membership and lower bacterial diversity in recipients compared to healthy transplant donors. High bacterial donor diversity was linked to decreased aGvHD risk	Liu et al. [63]
To analyze the impact of the fecal microbiota before allo-HSCT on clinical outcome of patients receiving HSCT	A prospective study, 16S rRNA sequencing of fecal samples	107 recipients undergoing allo-HSCT	aGVHD patients showed a significantly higher pre-HSCT abundance of Firmicutes and a lower level of Bacteroidetes in comparison with non-aGVHD patients	Doki et al. [65]
To examine the relationship between the abundance of microbiota species and relapse/progression of disease during 2 years of follow-up	A prospective study, 16S rRNA sequencing of fecal samples	541 patients undergoing allo-HSCT	A correlation between a higher abundance of *Eubacterium limosum* and a decreased risk of disease relapse/progression	Peled et al. [66]
To characterize the intestinal microbiota of GvHD and non-GvHD pediatric patients undergoing allo-HSCT	A prospective study, 16S rRNA sequencing of fecal samples	15 pediatric patients undergoing allo-HSCT	Pediatric patients developing GvHD had significantly higher antibiotic loads in comparison with non-GvHD patients	Simms-Waldrip et al. [74]
To monitor the changes in the microbiome among children patients post-allo-HSCT	A prospective study, 16S rRNA sequencing of fecal and oral samples	10 children receiving allo-HSCT	The data showed an impact of microbial metabolome on GvHD in post-HSCT pediatric patients	Parco et al. [64]
To investigate post-HSCT composition of gut microbiota	A prospective observational study, 16S rRNA sequencing of fecal samples	16 patients receiving allo-HSCT 8 patients after auto-HSCT	Differences in the intestinal microbiota of allo-HSCT patients, auto-HSCT recipients, and healthy controls. Proportions of *Bifidobacterium* were significantly lower in allo-HSCT patients compared to healthy controls	Kusakabe et al. [79]

Abbreviations: aGvHD, acute graft-versus-host disease; allo-HSCT, allogeneic hematopoietic stem cell transplantation; auto-HSCT, autologous hematopoietic stem cell transplantation; HLA, human leukocyte antigens; HSCT, hematopoietic stem cell transplantation; MDRO, multi-drug resistant organisms; OS, overall survival; rRNA, ribosomal ribonucleic acid.
The table summarizes the major findings from retrospective and prospective studies in hematologic cancer patients receiving HSCT.

improved survival linked to increased gut epithelial integrity and reduced GvHD after administration of butyrate-producing species of the class *Clostridia* [78]. A recent analysis from Kusakabe et al. confirmed the previously identified correlation between a low microbiota diversity in patients and a high frequency of complications and mortalityrate after transplantation. Moreover, significantly lower proportions of *Bifidobacterium* and butyrate-producing bacteria in allo-HSCT patients compared to healthy controls has been observed. Current findings suggest the important role of microbiota stability for the outcome of allo-HSCT patients [79].

A prospective single-center study of 131 adult patients undergoing allo-HSCT indicated a negative correlation between high urinary levels of 3-indoxyl sulfate produced by commensals (ie, *Lachnospiraceae* spp. and *Ruminococcaceae* spp.) and transplant-related mortality. Thereafter, 3-indoxyl sulfate might serve as a predictor, as its low urinary levels have been associated with gut microbiota disruption, treatment complications, and poor patient outcomes [80].

4.2. Autologous HSCT

Fewer data from the studies concerning the role of microbiota in autologous hematopoietic stem cell transplantation (auto-HSCT) patients are available (Table 2). In a recent study, Kusakabe with colleagues demonstrated substantial differences in gut microbiota between patients receiving allo-HSCT, healthy controls, and auto-HSCT recipients using weighted uniFrac distance analysis [79]. A single-center

retrospective trial analyzing 184 cancer patients undergoing auto-HSCT has evaluated the impact of colonization with multidrug-resistant organisms (MDRO) on overall survival. MDRO colonization was confirmed in 21.7% of patients. Non-relapse mortality (NRM) was statistically higher in MDRO-positive patients compared to the uncolonized group, representing 25.4% and 3%, respectively. While NRM in post-transplantation neutropenia did not differ between both groups, the authors pointed to the critical importance of the period after discharge from the hospital. Thus, MDRO colonized patients should be monitored in outpatient care and checked for the infections in the post-HSCT period [81].

A retrospective two-centers study analyzing microbiota diversity in a cohort of 365 cancer patients detected loss of diversity after auto-HSCT across both centers compared to healthy volunteers and public HMP datasets. Microbiome alterations between auto- and allo-HSCT patients showed that diversity decreased comparably between both groups. However, the microbiota of auto-HSCT patients recovered more rapidly after 30 days. Preliminary analysis suggested a correlation between lower diversity and shorter progression-free survival in myeloma patients undergoing auto-HSCT [82].

Currently, we have more data on the microbiome role in allo-opposite auto-HSCT, as the majority of studies are focused on allo-HSCT. Existing findings suggest, that the microbiome role in allo-HSCT is more complex, mainly due to the major effect of donor graft on the immune system, especially due to GvHD. However, we are still just at the beginning of understanding this process. For translation into the clinic, it is essential to capture comprehensively the relationship between microbiome, HSCT, and host variables, as there could be several unknown confounders that could influence the results of clinical trials, where only limited variables are taken into account. Larger trials, especially in auto-HSCT patients, with detailed characterization of multiple factors that could affect patients' microbiome, are warranted. Moreover, data from metagenomic analyses monitoring the long-term gut microbiome changes in patients receiving HSCT are still limited.

5. Therapeutic implications

Disruption of microbiota during cancer treatment increases the risk of secondary complications to a great extent. A growing body of experimental data has emerged the clinical potential of microbiota restoration [83], although its clinical use in oncology is still cautious considering the possible risk of further infection. Nevertheless, published results suggest that microbial modulation by fecal microbiota transplantation (FMT) or probiotics might improve the clinical outcomes of post-HSCT patients compared to the patients with no intervention to the gut microbiome (Fig. 2).

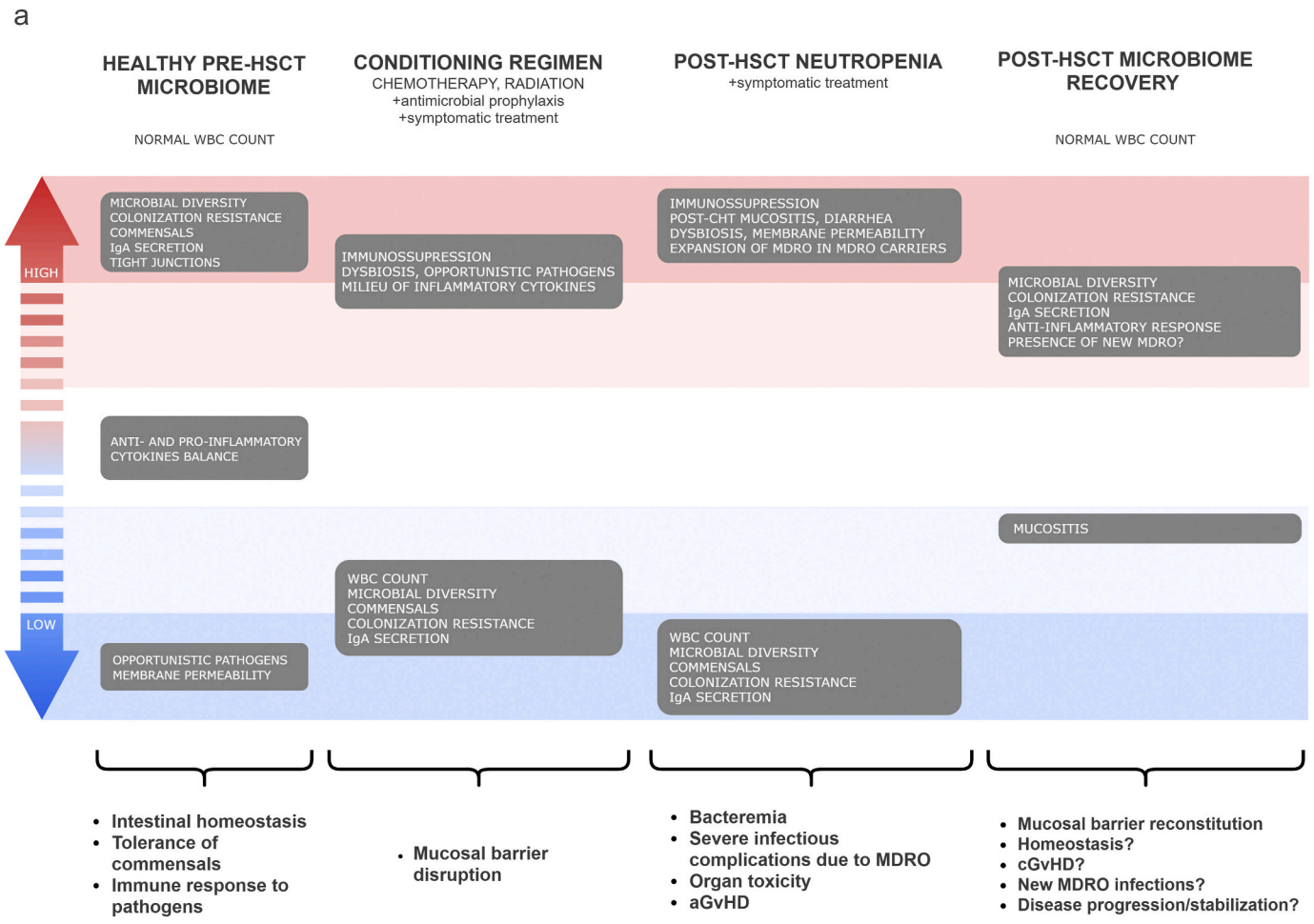


Fig. 2. A schematic overview of the link between gut microbiota changes (a) and the promising trend of FMT or probiotics modulation (b) in hematopoietic stem cell transplantation (HSCT). Microbiota restoration by FMT or probiotics might lead to an increased mucosal barrier reconstitution, eradication of MDRO, and a decrease of aGVHD and infectious complications in post-HSCT patients. A more pronounced color means that the relevant factor is higher (more red) or lower (more blue). *Abbreviations:* aGvHD, acute graft-versus-host disease; ATB, antibiotics; cGvHD, chronic graft-versus- host disease; CHT, chemotherapy; DFS, disease-free survival; FMT, fecal microbiota transplantation; HSCT, hematopoietic stem cell transplantation; IgA, Immunoglobulin A; MDRO, multi-drug resistant organisms; WBC, white blood cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

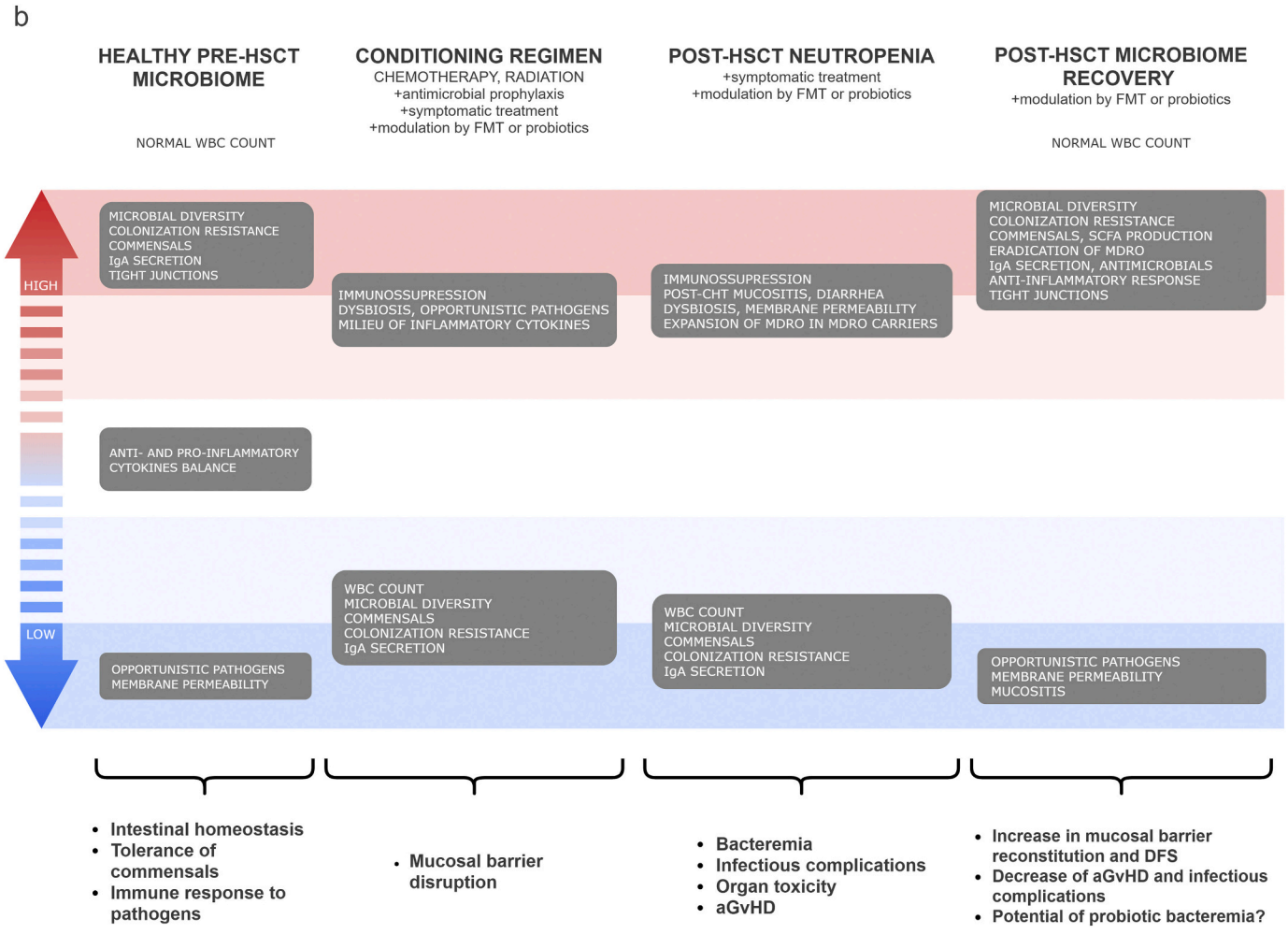


Fig. 2. (*continued*).

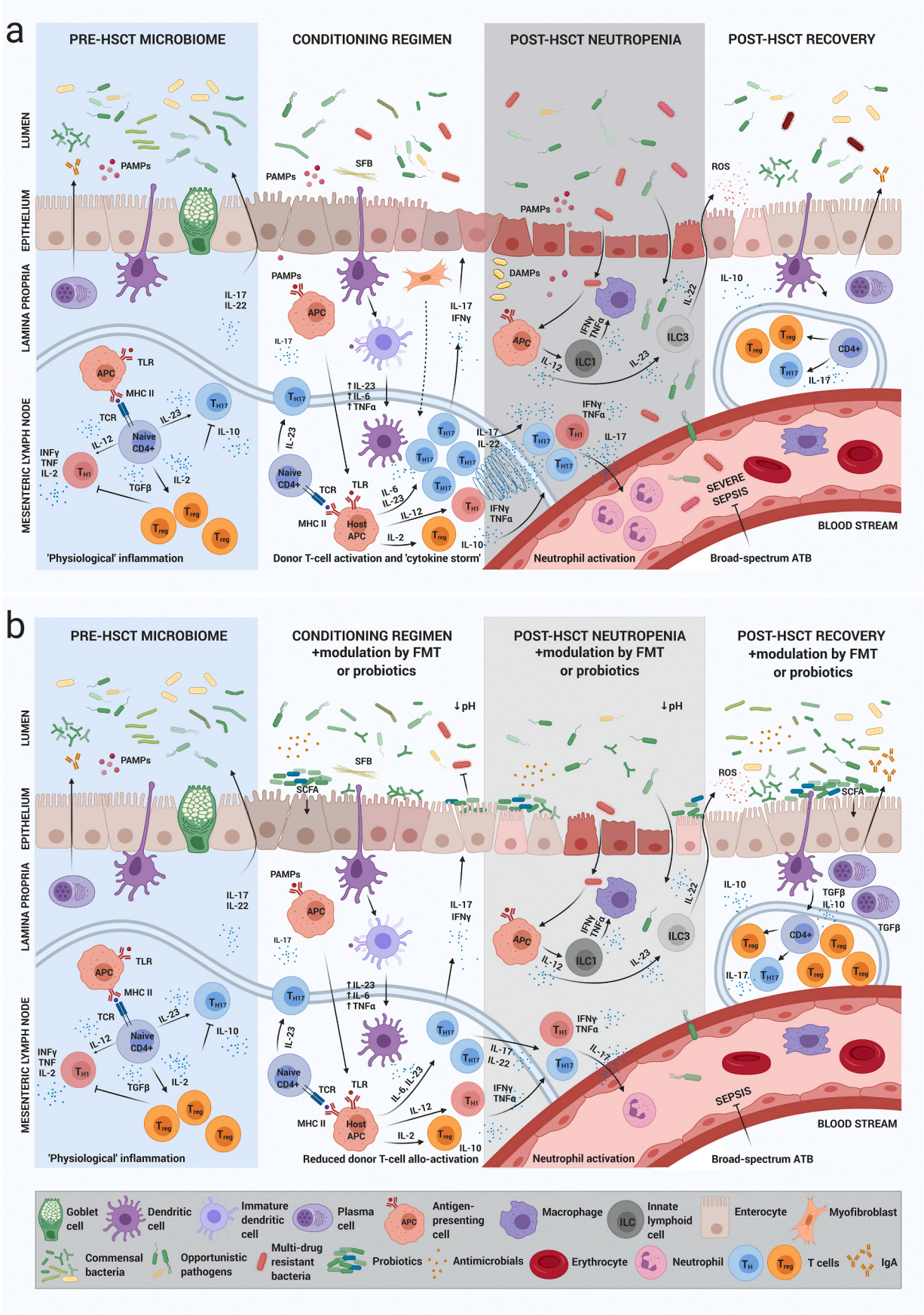
5.1. *Fecal microbiota transplantation*

FMT, representing a transfer of healthy donor stool to a recipient, has become a promising treatment of recurrent *Clostridium difficile* infections. The effectiveness of gut microbiota reconstitution after FMT is caused by competing with pathogens on one side, and also by microbial modulation of the recipient's immune system [84]. Murine models investigating the impact of FMT on reducing the toxicity of chemotherapy or radiation therapy showed the reconstitution of gut microbiota disrupted by cancer therapy to pretreatment state after FMT from untreated mice. Moreover, comparison of taxonomic and functional profiles did not reveal differences between pretreatment feces and samples collected one-week post-FMT [85,86]. Recent knowledge concerning the topic of efficacy, safety, clinical indications, and the best way of FMT delivery from a practical point of view is nicely reviewed in Krajicek et al. [87].

The loss of gut microbiota diversity during transplantation is associated with enhanced host immune response via inflammatory cytokines, BSI, and GvHD. However, the approach to reestablish depleted commensal bacteria by FMT or probiotics and prebiotics showed the encouraging results (Fig. 3).

Several small studies have demonstrated a beneficial effect of FMT on remission of GvHD in allo-HSCT patients. A case study with 4 patients has shown donor FMT was efficient in the therapy of acute intestinal steroid-refractory and -dependent GvHD [88]. Successful treatment of GvHD with encapsulated FMT confirmed the feasibility of this way of delivery [89]. Another pilot study consisting of 8 patients revealed that nasogastric tube delivery of donor FMT restored microbiota composition and increased diversity with the abundance of Bacteroidetes, Bacteroidaceae, Ruminococcaeae, and Desulfovibrionaceae in 6 patients. Consequently, patients undergoing FMT exhibited improved clinical symptoms regarding stool volume, abdominal pain, and achieved longer progression-free survival [90]. Similarly, analysis of gut microbiota compositional changes in 25 allo-HSCT patients (14 receiving autologous-FMT (auto-FMT) treatment and 11 controls) demonstrated that auto-FMT was an effective intervention restoring gut microbial diversity. In addition, reestablishing pre-allo-HSCT diversity and composition was associated with the reduction of transplant-related complications [91]. FMT was shown efficient also in the safe eradication of MDRO in a more recent study involving 10 allo-HSCT patients [92].

The small sample size and discrepancies in FMT delivery between reported studies are among the factors that have to be be taken into account when considering the safety and efficacy of FMT in patients receiving HSCT. Moreover, occasionally reported serious adverse events, force us to think about the potential risks of introducing live biotherapeutics. A recent report described two elderly patients with beta-lactamase (ESBL)–producing *Escherichia coli* bacteremia, transmitted from the same donor by FMT oral capsules in two different clinical trials. The first of them with advanced cirrhosis enrolled in a clinical trial concerning FMT in refractory hepatic encephalopathy treatment became clinically stable after a series of antibiotic regimens. However, the patient with myelodysplastic syndrome participating in a clinical trial on pre- and post- allo-HSCT administration of FMT capsules



(caption on next page)

Fig. 3. Gut microbiota and immune system crosstalk during hematopoietic stem cell transplantation (HSCT) in hematologic cancer patients. (a) In a patient with healthy pre-HSCT microbiota, plasma cells located in the lamina propria secrete IgA into the gut lumen. Dendritic cells (DCs) activated by microbiota subsequently migrate to the lymph nodes and change naive CD4+ T cells to regulatory or pro-inflammatory T cells (Tregs or Th17 cells, particularly). The maintenance of intestinal homeostasis is enabled by a delicate balance between secretion of anti- and pro-inflammatory cytokines (e.g. IL-10, TGFß, and IL-12, IL-17, IL-22, IL-23, IFNγ, respectively). Conditioning regimen including high dose chemotherapy, radiotherapy, as well as antimicrobial prophylaxis, and symptomatic treatment (PPI, antiemetics, laxatives) disrupt pre-HSCT gut microbial diversity leading to a massive pro-inflammatory immune response (cytokines storm) and mucositis. Moreover, injured epithelia secrete uric acid and adenosine triphosphate (ATP), activating antigen-presenting cells (APCs) resulting in the production of pro-inflammatory cytokines. In the early post-transplant period, the presence of neutropenia and mucosal barrier damage predisposes patients to severe infectious complications and sepsis. Translocation of luminal bacteria activates APCs by pathogen-associated molecular patterns (PAMPs) leading to donor T-cell activation and releasing of pro-inflammatory cytokines (IL-22, IL-17, IL-23) which aggravate acute GvHD (aGvHD). Particularly, bacteremia with MDRO is associated with an increase of both mortality and incidence of GvHD in allo-HSCT recipients. Interestingly, animal studies have shown recipient non-hematopoietic APCs are able to induce CD4+ T cell-dependent aGVHD as well. Broad-spectrum antibiotics in the early post-HSCT period can reduce the number of transmigrated bacteria in neutropenic patients, but the long-term administration leads to unfavorable effects due to the remarkable shift in microbial diversity and the risk of resistant pathogens selection. (b) Modulation of microbiota by FMT or prebiotics/probiotics reduce dysbiosis associated with poor post-transplantation outcomes. Probiotics help to boost microbial diversity and reverse the disruptive effects after conditioning regimen and broad-spectrum antibiotics, restore intestinal homeostasis via the production of antimicrobial substances and stimulation of anti-inflammatory cytokines secretion. Probiotic bacteria alter the DCs in a way to support Treg over Th17 phenotype. Moreover, beneficial bacteria provide an energy source for enterocytes via SCFA production leading to the improvement of barrier integrity. Altering luminal pH results in an unfavorable environment for pathogen colonization. Importantly, antibiotic-resistant bacterial strains might be diminished by FMT or prebiotics/probiotics via inhibition of pathogen adhesion and competition for nutrients (the results are promising mainly regarding Gram-positive organisms). Therefore, the reestablishment of depleted commensal bacteria might help to achieve the optimal therapeutic outcome, while decreasing the risk of severe post-transplant complications as bacteremias and GvHD.

Abbreviations: aGvHD, acute graft-versus-host disease; allo-HSCT, allogeneic hematopoietic stem cell transplantation; APC, antigen-presenting cell; ATB, antibiotics; ATP, adenosine triphosphate; DAMPs, damage-associated molecular patterns; DC, dendritic cell; FMT, fecal microbiota transplantation; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; IFNγ, interferon-gamma; IgA, immunoglobulin A; IL, interleukin; MDRO, multi-drug resistant organisms; MHC class II, major histocompatibility complex class II; PAMPs, pathogen-associated molecular patterns; PPI, proton-pump inhibitor; ROS, reactive oxygen species; SCFA, short-chain fatty acid; SFB, segmented filamentous bacteria; T cells, a type of lymphocyte developing in the thymus; TCR, the T cell receptor; TGFß, transforming growth factor-beta; Th17 cells, T helper cells; TLR, toll-like receptor; TNFα, tumor necrosis factor-alpha; T reg cells, regulatory T cells.

died from severe sepsis a week after stem-cell infusion, 10 days after final FMT dose [93]. These findings undoubtedly highlight the crucial need for more comprehensive donor-screening practices and careful benefit-risk assessment in the study design. In this context, randomized control trials investigating the impact of FMT in large cohorts are needed for further evaluations. Currently, several ongoing trials evaluate the impact of FMT on post-HSCT patient therapy (Table 3), and some results have been already published [94].

5.2. Probiotics and prebiotics

Probiotics are viable organisms for healthy gut restoration. Their administration in cancer patients is common and primarily focused on the mitigation of adverse effects of treatment. A single-center probiotic survey among 499 cancer patients reported probiotic use in 28,5% of all respondents [95]. Side effects of multiple chemotherapeutics were shown to be directly associated with gut microbiota. Some metabolites of chemotherapeutic agents affect the intestinal mucosa leading to severe diarrhea [96]. For instance, the colon cancer drug irinotecan is converted to the active metabolite SN-38 by beta-glucuronidase produced by commensal bacteria in the intestine, worsening treatment-associated diarrhea through epithelial barrier damage and mucositis [97–99]. Guthrie et al. demonstrated that specific bacterial ß-glucuronidases and transporters were in correlation with a distinct SN-38G reactivation studied ex vivo in healthy individuals [100]. Importantly, we observed reduced gastrointestinal toxicity in terms of incidence and severity, after modification of gut microbiota by probiotics in colon cancer patients treated with irinotecan [101].

Preclinical and clinical studies of probiotic usage in HSCT patients have shown inconsistent clinical outcomes. Therefore, patients' unique microbiota variability should be taken into account to overcome the resistance to probiotic colonization, a major contributing factor of insufficient probiotic efficacy in many cases. Administration of the probiotic *Lactobacillus rhamnosus* GG reduced the incidence of aGvHD in mouse models [102]. Nevertheless, the first randomized clinical trial on allo-HSCT patients supplemented with *Lactobacillus rhamnosus* GG confirmed neither probiotic-related associations nor changes in the incidence of GvHD, and had to be terminated [103]. The possibility of bacterial translocation followed by *Lactobacillus* bacteremia represents one of the major concerns for probiotic administration in immunocompromised patients [104]. The risk of bacteremia might be even greater in HSCT patients with mucosal barrier disruption. A study evaluating the safety and feasibility of *Lactobacillus plantarum* (LBP) in pediatric patients undergoing allo-HSCT reported no case of LBP bacteremia through the first 14 days post-HSCT [105]. Furthermore, a large retrospective analysis of HSCT patients receiving probiotic supplementation showed that 19 of 3796 (0.5%) patients developed a BSI, mostly from *Lactobacillus* spp. These data suggest that available probiotic supplements appear to be rarely associated with BSI and mortality after HSCT [106]. Recently, a single-center retrospective study supported the safety of probiotics in high-risk pediatric allo-HSCT patients, with no patients developing BSI with *Lactobacillus* or other probiotic strains [107].

In comparison with probiotics, dietary fibers fermented by gut microbiota (such as starches, fructooligosaccharides, and galactooligosaccharides) commonly known as prebiotics, alter microbiota composition while minimizing the risk of bacteremia in severely immunocompromised populations. A retrospective cohort study in HSCT patients reported that the combination of glutamine, fiber, and a fructooligosaccharide effectively decreased the severity of mucosal damage post-transplant. However, no effect on GvHD has been detected. In patients receiving prebiotics, reduction in days of diarrhea grade 3–4, as well as in days of mucositis grade 3–4 were observed. Accordingly, survival at day 100, weight loss, and the number of intravenous hyperalimentation days were better in patients on prebiotics compared to those who did not receive the supplementation. It is worth mentioning, the patients enrolled also received *Lactobacillus* spp. [108]. The results of two clinical trials, specifically NCT027630331 investigating the association between potato starch and risk of GvHD, and NCT02805075 determining the tolerability of HSCT patients to fructooligosaccharides are still awaited [109].

6. Conclusions and future directions

Nowadays, there is considerable evidence for the effect of the microbiome in carcinogenesis and cancer treatment. An ample amount of published data suggests that an individual's response to cancer therapy might be linked with the microbiome via reciprocal interaction

Table 3
Therapeutic implication of Fecal Microbiota Transplantation (FMT) in Hematopoietic Stem Cell Transplantation (HSCT).

Study	Study design	Disease	Purpose	Patients (n)	Intervention	Study status
NCT02733744	A pilot interventional study	Allo-HSCT recipients	To determine the feasibility of FMT in capsule form and its clinical benefits	18 adults	FMT from healthy donors will be given orally in 30 capsules (15 capsules daily x 2 consecutive days) in the first 3 weeks after recovery of white blood cells after HSCT	Completed
Study results: "The study indicates that empiric third-party FMT after allo-HCT appears to be feasible, safe, and associated with the expansion of recipient microbiome diversity." [94]						
NCT03862079	A randomized phase 2 trial	Allo-HSCT recipients receiving the broad-spectrum antibiotics	To investigate if FMT with or without total gut decontamination works in preventing GvHD	120 adults	Standardly treated patients will be compared to patients in the experimental arm I who will receive piperacillin/tazobactam and nystatin orally than FMT via enema after HSCT and in the experimental arm II solo FMT	Withdrawn
NCT03678493	A randomized, double-blind, placebo-controlled phase 2 study	Acute myeloid leukemia patients receiving intensive chemotherapy, and allo-HSCT patients	To assess the efficacy of capsule form of FMT on the incidence of infections, aGvHD, rate of engraftment of FMT	120 adults	Participants will receive up to 3 treatments of FMT vs. placebo after antibiotic therapy until 3 months post-randomization	Recruiting
NCT03214289	An interventional phase 1 study	Patients with steroid-resistant or steroid-dependent gut aGvHD	To evaluate the safety and feasibility of FMT with frozen capsules from healthy donors	4 adults	FMT will be given through the ingestion of 30 capsules (15 capsules daily × 2 consecutive days) in the experimental arm	Unknown
NCT03359980	An interventional phase 2 study	Patients with steroid-resistant gut aGvHD	To investigate the efficacy of FMT	32 adults	Transfer of fecal microbiota from healthy donors	Active, not recruiting
NCT03492502	An Interventional single-arm study	Patients with acute steroid-resistant gut GvHD	To assess the safety and efficacy of autologous FMT	70 adults	Autologous FMT by nasogastric tube will be given once daily for two consecutive days	Withdrawn (slow recruitment)
NCT03549676	A pilot single-arm open label study	Allo-HSCT patients with acute steroid-resistant GI-related GvHD	To assess the safety and efficacy of FMT	15 children	Patients will receive FMT from unrelated healthy donors through a nasojejunal tube, a second-time FMT using a different donor should be considered for patients without a respond	Not yet recruiting
NCT03720392	A interventional randomized phase 2 study	Allo-HSCT recipients	To examine the microbial flora diversity after FMT administration and incidence rate of GvHD and other post-HSCT complications	48 adults	Oral FMT in capsules before and after HSCT	Active, not recruiting
NCT02398708	An observational, case-control, cross-sectional study	Allo-HSCT recipients with and without cGvHD	To estimate inflammatory markers, diversity, and levels of fecal microbes of individuals post-allo-HSCT	38 adults	/	Completed – results not posted
NCT02269150	A randomized, open-label, controlled study	Hematologic malignancies, allo-HSCT recipients	To see if autologous FMT will prevent the future development of CDI	96 adults	Experimental arm: Patients undergo FMT with the pre-transplantation during the engraftment phase	Active, not recruiting

Abbreviations: aGvHD – acute graft-versus-host disease; allo-HSCT, allogeneic hematopoietic stem cell transplantation; CDI, *Clostridium difficile* Infection; cGvHD, chronic graft-versus-host disease; FMT, fecal microbiota transplantation; GI, gastrointestinal; HSCT, hematopoietic stem cell transplantation.
The table summarizes the list of ongoing and completed clinical trials dealing with the safety and efficacy of FMT and its impact on microbial diversity, incidence rate of GvHD and other complications in post-HSCT patients (according to https://clinicaltrials.gov/).

between host and environmental factors. Specific bacterial species can potentiate the immune system and are associated with a favorable tumor-immune microenvironment. Currently, a connection between particular changes in microbiota composition and post-HSCT complications has been documented. Microbial therapy that both promotes anticancer treatment and reduces systemic toxicity taking into account the specific microbiome of cancer patients, influenced by their lifestyle and other factors, could help clinicians to select the patients for particular treatment modality. However, metagenomic analyses monitoring the microbiome in the long-term are still needed. Detailed investigation of both the microbiome and the host immune system may help find microbiome markers useful for early identification of patients with a higher risk of post-transplant complications, particularly GvHD. This might make it possible to modulate the gut microbiota via targeted antibiotics, the use of probiotics and prebiotics, genetically modified phages, or FMT in a patient-specific manner. According to encouraging results, targeted modification of the intestinal immune system is likely to be used as a future trend in standard care for HSCT patients to achieve optimal therapeutic outcomes and follow-up, while avoiding severe transplant-related complications.

Practice points

- Growing evidence highlighting the critical role of microbiome composition in hematologic cancer patients receiving HSCT.
- Conditioning regimens disturb the homeostatic dialog between the gut microbiota and the host immune system leading to intestinal dysbiosis.
- Mucosal barrier disruption and reduced microbial diversity are associated with severe infectious complications and GvHD.
- Long-term prophylaxis with broad-spectrum antibiotics causes remarkable shifts in microbiome composition and losses of microbial diversity leading to unfavorable side effects.
- Gut microbiota modulation by FMT or probiotics might be an option to improve the clinical outcomes of hematologic cancer patients.

Research agenda

- Due to the limiting data, further research is warranted to evaluate the associations between the microbiome changes and post-transplant complications in auto-HSCT patients.

- The impact of microbiota alterations on host immune system cross-talk via cytokine levels, as well as the studies of liquid biopsy biomarkers such as miRNA, cell-free DNA in HSCT patients are encouraged.
- Metagenomic analyses monitoring the long-term gut microbiome changes in patients receiving HSCT are still needed.
- Clinical trials concerning the safety and efficacy of gut microbiota modulation by probiotics and prebiotics or FMT with a larger number of participants are highly recommended.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We would greatly thank Mr. Jaroslav Gasinec Jr. for his wonderful help with the graphical visualization of the abstract and final graphical format of pictures and tables. Moreover, we would like to thank Mrs. Rebecca Doherty and Dr. Richard Kracer for reading the manuscript carefully and helping with language editing. Fig. 3 was prepared with BioRender.com.

Role of the funding source

This work was supported by Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and Slovak Academy of Sciences VEGA, project numbers 2/0052/18 and 1/0327/19. The funding source had no influence on the writing of the manuscript.

References

[1] Grice EA, Segre JA. The human microbiome: our second genome. Annu Rev Genomics Hum Genet 2012;13:151–70.
[2] Lederberg J, McCray AT. Ome sweet omics—a genealogical treasury of words. Scientist 2001;15(7). 8.
[3] Goodrich JK, Di Rienzi SC, Poole AC, Koren O, Walters WA, Caporaso JG, et al. Conducting a microbiome study. Cell 2014;158(2):250–62.
[4] Geva-Zatorsky N, Sefik E, Kua L, Pasman L, Tan TG, Ortiz-Lopez A, et al. Mining the human gut microbiota for immunomodulatory organisms. Cell. 2017;168(5):928–43.
[5] Korem T, Zeevi D, Suez J, Weinberger A, Avnit-Sagi T, Pompan-Lotan M, et al. Growth dynamics of gut microbiota in health and disease inferred from single metagenomic samples. Science 2015;349(6252):1101–6.
[6] Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, et al. Environment dominates over host genetics in shaping human gut microbiota. Nature 2018;555(7695):210–5.
[7] Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010;464(7285):59–65.
[8] The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486(7402):207–14.
[9] Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 2019;7(1):14.
[10] Feng Q, Chen WD, Wang YD. Gut microbiota: an integral moderator in health and disease. Front Microbiol 2018;9:151.
[11] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005;308(5728):1635–8.
[12] Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature 2011;473(7346):174–80.
[13] Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature 2012;486(7402):222–7.
[14] De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107(33):14691–6.
[15] Takeshita T, Matsuo K, Furuta M, Shibata Y, Fukami K, Shimazaki Y, et al. Distinct composition of the oral indigenous microbiota in South Korean and Japanese adults. Sci Rep 2014;4:6990.
[16] Tyakht AV, Kostryukova ES, Popenko AS, Belenikin MS, Pavlenko AV, Larin AK, et al. Human gut microbiota community structures in urban and rural populations in Russia. Nat Commun 2013;4:2469.
[17] Zhang J, Guo Z, Xue Z, Sun Z, Zhang M, Wang L, et al. A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. ISME J 2015;9(9):1979–90.
[18] Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, et al. An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol 2014;32(8):834–41.
[19] Mego M, Čierniková S, Razus M, Drgoňa Ľ, Zajac V. Probiotic bacteria in patients treated with chemotherapy and radiation therapy. In: Ullah MF, Ahmad A, editors. Critical dietary factors in cancer chemoprevention. Springer International Publishing; 2016. p. 353–73.
[20] Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. Biol Blood Marrow Transplant 2009;15(3):367–9.
[21] Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood 2014;124(3):344–53.
[22] Koyama M, Kuns RD, Olver SD, et al. Recipient nonhematopoietic antigen-presenting cells are sufficient to induce lethal acute graft-versus-host disease. Nat Med 2011;18(1):135–42.
[23] Hill BT, Rybicki L, Bolwell BJ, Smith S, Dean R, Kalaycio M, et al. The non-relapse mortality rate for patients with diffuse large B-cell lymphoma is greater than relapse mortality 8 years after autologous stem cell transplantation and is significantly higher than mortality rates of population controls. Br J Haematol 2011;152(5):561–9.
[24] Martin PJ, Counts Jr GW, Appelbaum FR, Lee SJ, Sanders JE, Deeg HJ, et al. Life expectancy in patients surviving more than 5 years after hematopoietic cell transplantation. J Clin Oncol 2010;28(6):1011–6.
[25] Wingard JR, Majhail NS, Brazauskas R, Wang Z, Sobocinski KA, Jacobsohn D, et al. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. J Clin Oncol 2011;29(16):2230–9.
[26] Zackular JP, Baxter NT, Iverson KD, Sadler WD, Petrosino JF, Chen GY, et al. The gut microbiome modulates colon tumorigenesis. mBio 2013;4(6). e00692-13.
[27] Roy S, Trinchieri G. Microbiota: a key orchestrator of cancer therapy. Nat Rev Cancer 2017;17(5):271–85.
[28] Goubet AG, Daillère R, Routy B, Derosa LM, Roberti P, Zitvogel L. The impact of the intestinal microbiota in therapeutic responses against cancer. C R Biol 2018;341(5):284–9.
[29] Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013;342(6161):971–6.
[30] Kim YS, Kim J, Park SJ. High-throughput 16S rRNA gene sequencing reveals alterations of mouse intestinal microbiota after radiotherapy. Anaerobe 2015;33C:1–7.
[31] Manichanh C, Varela E, Martinez C, Antolin M, Llopis M, Dore J, et al. The gut microbiota predispose to the pathophysiology of acute postradiotherapy diarrhea. Am J Gastroenterol 2008;103:1754–61.
[32] Gerassy-Vainberg S, Blatt A, Danin-Poleg Y, Gershovich K, Sabo E, Nevelsky A, et al. Radiation induces proinflammatory dysbiosis: transmission of inflammatory susceptibility by host cytokine induction. Gut 2018;67(1):97–107.
[33] Nam YD, Kim HJ, Seo JG, Kang SW, Bae JW. Impact of pelvic radiotherapy on gut microbiota of gynecological cancer patients revealed by massive pyrosequencing. PLoS One 2013;8(12):e82659.
[34] Wang A, Ling Z, Yang Z, Kiela PR, Wang T, Wang C, et al. Gut microbial dysbiosis may predict diarrhea and fatigue in patients undergoing pelvic cancer radiotherapy: a pilot study. PLoS One 2015;10(5):e0126312.
[35] Wang Z, Wang Q, Wang X, Zhu L, Chen J, Zhang B, et al. Gut microbial dysbiosis is ssociated with development and progression of radiation enteritis during pelvic radiotherapy. J Cell Mol Med 2019;23(5):3747–56.
[36] Ferreira MR, Jervoise H, Andreyev N, Mohammed K, Truelove L, Gowan SM, et al. Microbiota- and radiotherapy-induced gastrointestinal side-effects (MARS) study: a large pilot study of the microbiome in acute and late-radiation enteropathy. Clin Cancer Res 2019;25(21):6487–500.
[37] Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat Rev Gastroenterol Hepatol 2017;14(6):356–65.
[38] Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013;342:967–70.
[39] Gui QF, Lu HF, Zhang CX, Xu ZR, Yang YH. Well-balanced commensal microbiota contributes to anti-cancer response in a lung cancer mouse model. Genet Mol Res 2015;14:5642–51.
[40] Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 2015;372(4):311–9.
[41] Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373(17):1627–39.
[42] Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. JAMA 2016;315(15):1600–9.
[43] Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015;350(6264):1079–84.
[44] Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 2015;350(6264):1084–9.

[45] Mager LF, Burkhard R, Pett N, Cooke NCA, Brown K, Ramay H, et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. Science 2020;369(6510):1481–9.
[46] Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018;359(6371):91–7.
[47] Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018;359(6371):97–103.
[48] Balletto E, Mikulska M. Bacterial infections in hematopoietic stem cell transplant recipients. Mediterr J Hematol Infect Dis 2015;7(1):e2015045.
[49] Almyroudis NG, Fuller A, Jakubowski A, Sepkowitz K, Jaffe D, Small TN, et al. Pre- and post-engrafment bloodstream infection rates and associated mortality in allogeneic hematopoietic stem cell transplant recipients. Transpl Infect Dis 2005; 7:11–7.
[50] Blennow O, Ljungman P, Sparrelid E, Mattsson J, Remberger M. Incidence, risk factors, and outcome of bloodstream infections during the pre-engrafment phase in 521 allogeneic hematopoietic stem cell transplantations. Transpl Infect Dis 2014;16:106–14.
[51] Mikulska M, Del Bono V, Bruzzi P, Raiola AM, Gualandi F, Van Lint MT, et al. Mortality afer bloodstream infections in allogeneic haematopoietic stem cell transplant (HSCT) recipients. Infection 2012;40:271–8.
[52] Sommer MO, Dantas G, Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. Science 2009;325(5944):1128–31.
[53] Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol 2008;6(11):e280.
[54] Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A 2011;108(1):4554–61.
[55] Alexander S, Fisher BT, Gaur AH, Dvorak CC, Villa Luna D, Dang H, et al. Effect of levofloxacin prophylaxis on bacteremia in children with acute leukemia or undergoing hematopoietic stem cell transplantation: a randomized clinical trial. JAMA 2018 Sep 11;320(10):995–1004.
[56] Stoma I, Karpov I, Iskrov I, Krivenko S, Uss A, Vlasenkova S, et al. Decolonization of intestinal carriage of MDR/XDR gram-negative bacteria with oral colistin in patients with hematological malignancies: results of a randomized controlled trial. Mediterr J Hematol Infect Dis 2018;10(1):e2018030.
[57] Vehreschild JJ, Moritz G, Vehreschild MJ, Arenz D, Mahne M, Bredenfeld H, et al. Efficacy and safety of moxifloxacin as antibacterial prophylaxis for patients receiving autologous haematopoietic stem cell transplantation: a randomised trial. Int J Antimicrob Agents 2012;39(2):130–4.
[58] Ferrara JL, Smith CM, Sheets J, Reddy P, Serody JS. Altered homeostatic regulation of innate and adaptive immunity in lower gastrointestinal tract GVHD pathogenesis. J Clin Invest 2017;127(7):2441–5.
[59] Bilinski J, Robak K, Peric Z, Marchel H, Karakulska-Prystupiuk E, Halaburda K, et al. Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of allogeneic hematopoietic stem cell transplantation: a retrospective, single-center study. Biol Blood Marrow Transplant 2016;22(6):1087–93.
[60] Kaysen A, Heintz-Buschart A, Muller EE, Narayanasamy S, Wampach L, Laczny CC, et al. Integrated meta-omic analyses of the gastrointestinal tract microbiome in patients undergoing allogeneic hematopoietic stem cell transplantation. Transl Res 2017;186:79–94.
[61] Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased GVHDrelated mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl Med 2016;8(339). 339ra71.
[62] Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood 2014;124(7):1174–82.
[63] Liu C, Frank DN, Horch M, Chau S, Ir D, Horch EA, et al. Associations between acute gastrointestinal GvHD and the baseline gut microbiota of allogeneic hematopoietic stem cell transplant recipients and donors. Bone Marrow Transplant 2017;52(12):1643–50.
[64] Parco S, Benericetti G, Vascotto F, Palmisciano G. Microbiome and diversity indices during blood stem cells transplantation – new perspectives? Cent Eur J Public Health 2019;27(4):335–9.
[65] Doki N, Suyama M, Sasajima S, Ota J, Igarashi A, Mimura I, et al. Clinical impact of pretransplant gut microbial diversity on outcomes of allogeneic hematopoietic stem cell transplantation. Allo-HSCT may be a strategy to prevent aGVHD. Ann Hematol 2017;96(9):1517–23.
[66] Peled JU, Devlin SM, Staffas A, Lumish M, Khanin R, Littmann ER, et al. Intestinal microbiota and relapse after hematopoietic-cell transplantation. J Clin Oncol 2017;35(15):1650–9.
[67] Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal Blautia is associated with reduced death from graft-versus-host disease. Biol Blood Marrow Transplant 2015;21(8):1373–83.
[68] Peled JU, Gomes ALC, Devlin SM, Littmann ER, Taur Y, Sung AD, et al. Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. N Engl J Med 2020;382(9):822–34.
[69] Köhler N, Zeiser R. Intestinal microbiota influence immune tolerance post allogeneic hematopoietic cell transplantation and intestinal GVHD. Front Immunol 2019;9:3179.
[70] Holler E, Butzhammer P, Schmid K, Hundsrucker C, Koestler J, Peter K, et al. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. Biol Blood Marrow Transplant 2014;20(5):640–5.
[71] Stein-Thoeringer CK, Nichols KB, Lazrak A, Docampo MD, Slingerland AE, Slingerland JB, et al. Lactose drives *Enterococcus* expansion to promote graft-versus-host disease. Science 2019;366(6469):1143–9.
[72] Kusakabe S, Fukushima K, Yokota T, Hino A, Fujita J, Motooka D, et al. *Enterococcus*: a predictor of ravaged microbiota and poor prognosis after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2020;26(5):1028–33.
[73] Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes 2016;7(3):189–200.
[74] Simms-Waldrip TR, Sunkersett G, Coughlin LA, Savani MR, Arana C, Kim J, et al. Antibiotic-induced depletion of anti-inflammatory *Clostridia* is associated with the development of GVHD in pediatric stem cell transplant patients. Biol Blood Marrow Transplant 2017;23:820–9.
[75] Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab 2011;13(5):517–26.
[76] Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc Natl Acad Sci U S A 2014;111(6):2247–52.
[77] Mathewson ND, Jenq R, Mathew AV, Koenigsknecht M, Hanash A, Toubai T, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. Nat Immunol 2016;17(5):505–13.
[78] Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. Nature 2013;500(7461):232–6.
[79] Kusakabe S, Fukushima K, Maeda T, Motooka D, Nakamura S, Fujita J, et al. Pre- and post-serial metagenomic analysis of gut microbiota as a prognostic factor in patients undergoing haematopoietic stem cell transplantation. Br J Haematol 2020;188(3):438–49.
[80] Weber D, Oefner PJ, Hiergeist A, Koestler J, Gessner A, Weber M, et al. Low urinary indoxyl sulfate levels early after transplantation reflect a disrupted microbiome and are associated with poor outcome. Blood 2015;126(14):1723–8.
[81] Scheich S, Reinheimer C, Brandt C, Wichelhaus TA, Hogardt M, Kempf VA, et al. Clinical impact of colonization with multidrug-resistant organisms on outcome after autologous stem cell transplantation: a retrospective single center study. Biol Blood Marrow Transplant 2017;23(9):1455–62.
[82] Khan N, Peled JU, Gomes ALC, Devlin AM, Rondon-Clavo C, Clurman A, et al. Loss of microbiota diversity after autologous stem cell transplant is comparable to injury in allogeneic stem cell transplant. Blood 2018;132(Supplement 1). 608.
[83] Ciernikova S, Mego M, Hainova K, Adamcikova Z, Stevurkova V, Zajac V. Modification of microflora imbalance: future directions for prevention and treatment of colorectal cancer? Neoplasma 2015;62(3):345–52.
[84] Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. Nat Rev Gastroenterol Hepatol 2016;13(9):508–16.
[85] Cui M, Xiao H, Li Y, Zhou L, Zhao S, Luo D, et al. Faecal microbiota transplantation protects against radiation-induced toxicity. EMBO Mol Med 2017; 9(4):448–61.
[86] Le Bastard Q, Ward T, Sidiropoulos D, Hillmann BM, Chun CL, Sadowsky MJ, et al. Fecal microbiota transplantation reverses antibiotic and chemotherapy-induced gut dysbiosis in mice. Sci Rep 2018;8(1):6219.
[87] Krajicek E, Fischer M, Allegretti JR, Kelly CR. Nuts and bolts of fecal microbiota transplantation. Clin Gastroenterol Hepatol 2019;17(2):345–52.
[88] Kakihana K, Fujioka Y, Suda W. Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. Blood 2016;128(16): 2083–8.
[89] Kaito S, Toya T, Yoshifuji K. Fecal microbiota transplantation with frozen capsules for a patient with refractory acute gut graft-versus-host disease. Blood Adv 2018;2(22):3097–101.
[90] Qi X, Li X, Zhao Y. Treating steroid refractory intestinal acute graft-vs-host disease with fecal microbiota transplantation: a pilot study. Front Immunol 2018; 9:2195.
[91] Taur Y, Coyte K, Schluter J, Robilotti E, Figueroa C, Gjonbalaj M, et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. Sci Transl Med 2018;10(460). eaap9489.
[92] Battipaglia G, Malard F, Rubio MT. Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematological malignancies carrying multidrug-resistance bacteria. Haematologica 2019;104 (8):1682–8.
[93] DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. N Engl J Med 2019;381(21):2043–50.
[94] DeFilipp Z, Peled JU, Li S, Mahabamunuge J, Dagher Z, Slingerland AE, et al. Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity. Blood Adv 2018;2(7):745–753.95.
[95] Ciernikova S, Mego M, Semanova M, Wachsmannova L, Adamcikova Z, Stevurkova V, et al. Probiotic survey in cancer patients treated in the outpatient department in a comprehensive cancer center. Integr Cancer Ther 2017;16(2): 188–95.
[96] Mego M, Holec V, Drgona L, Hainova K, Ciernikova S, Zajac V. Probiotic bacteria in cancer patients undergoing chemotherapy and radiation therapy. Complement Ther Med 2013;21(6):712–23.
[97] Lin XB, Farhangfar A, Valcheva R, Sawyer MB, Dieleman L, Schieber A, et al. The role of intestinal microbiota in development of irinotecan toxicity and in toxicity reduction through dietary fibres in rats. PLoS One 2014;9(1):e83644.

[98] Logan RM, Gibson RJ, Bowen JM, Stringer AM, Sonis ST, Keefe DM. Characterisation of mucosal changes in the alimentary tract following administration of irinotecan: implications for the pathobiology of mucositis. Cancer Chemother Pharmacol 2008;62(1):33–41.

[99] Stringer AM, Gibson RJ, Bowen JM, Logan RM, Ashton K, Yeoh AS, et al. Irinotecan-induced mucositis manifesting as diarrhoea corresponds with an amended intestinal flora and mucin profile. Int J Exp Pathol 2009;90(5):489–99.

[100] Guthrie L, Gupta S, Daily J, Kelly L. Human microbiome signatures of differential colorectal cancer drug metabolism. NPJ Biofilms Microbiomes 2017;3:27.

[101] Mego M, Chovanec J, Vochyanova-Andrezalova I, Konkolovsky P, Mikulova M, Reckova M, et al. Prevention of irinotecan induced diarrhea by probiotics: a randomized double blind, placebo controlled pilot study. Complement Ther Med 2015;23(3):356–62.

[102] Gerbitz A, Schultz M, Wilke A, Linde HJ, Schölmerich J, Andreesen R, et al. Probiotic effects on experimental graft-versus-host disease: let them eat yogurt. Blood 2004;103(11):4365–7.

[103] Gorshein E, Wei C, Ambrosy S, Budney S, Vivas J, Shenkerman A, et al. Lactobacillus rhamnosus GG probiotic enteric regimen does not appreciably alter the gut microbiome or provide protection against GVHD after allogeneic hematopoietic stem cell transplantation. Clin Transplant 2017;31(5):e12947.

[104] Mehta A, Rangarajan S, Borate U. A cautionary tale for probiotic use in hematopoietic SCT patients-*Lactobacillus acidophilus* sepsis in a patient with mantle cell lymphoma undergoing hematopoietic SCT. Bone Marrow Transplant 2013;48(3):461–2.

[105] Ladas EJ, Bhatia M, Chen L, Sandler E, Petrovic A, Berman DM, et al. The safety and feasibility of probiotics in children and adolescents undergoing hematopoietic cell transplantation. Bone Marrow Transplant 2016;51(2):262.

[106] Cohen SA, Woodfield MC, Boyle N, Stednick Z, Boeckh M, Pergam SA. Incidence and outcomes of bloodstream infections among hematopoietic cell transplant recipients from species commonly reported to be in over-the-counter probiotic formulations. Transpl Infect Dis 2016;18(5):699–705.

[107] Sadanand A, Newland JG, Bednarski JJ. Safety of probiotics among high-risk pediatric hematopoietic stem cell transplant recipients. Infect Dis Ther 2019;8(2):301–6.

[108] Iyama S, Sato T, Tatsumi H, Hashimoto A, Tatekoshi A, Kamihara Y, et al. Efficacy of enteral supplementation enriched with glutamine, fiber, and oligosaccharide on mucosal injury following hematopoietic stem cell transplantation. Case Rep Oncol 2014;7(3):692–9.

[109] Severyn CJ, Brewster R, Andermann TM. Microbiota modification in hematology: still at the bench or ready for the bedside? Blood Adv 2019;3(21):3461–72.