HHS Public Access

Author manuscript

Biol Blood Marrow Transplant. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

Biol Blood Marrow Transplant. 2018 July ; 24(7): 1322–1340. doi:10.1016/j.bbmt.2018.02.009.

The Microbiome and Hematopoietic Cell Transplantation: Past, Present, and Future

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Conflicts of Interest

No conflicts of interest to report: TMA, CH, PR, MLR, RS, TT, AA, SB, PC, WBC, EH, AH, AYK, PLM, JMM, RN, KR, BES, EJS, ADS, DW, JW, JRW, WAW, MAP, ASB

Seres Therapeutics Inc: JUP (licensing fees and research support), MRMVDB (research support), RRJ (advisory committee, holds patents with or receives royalties)

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Keywords

Microbiome; Microbiota; Hematopoietic stem cell transplantation; Metagenomics; Prebiotic; Fecal microbiota transplant

Introduction

The human microbiome is comprised of the bacteria, archaea, viruses, fungi and other microeukaryotes that live on and within the human host. Alterations in the microbiome are associated with adverse transplant outcomes including the expected infectious complications following allogeneic hematopoietic cell transplantation (allo-HCT) in addition to diseases that are not classically "microbe-associated". For example, recent data suggest an association between certain microbial community structures and mortality, disease relapse, risk of infection, and graft-versus-host disease (GVHD)[1,2,3]. Most studies inspecting the role of the microbiome in HCT patient outcomes, though compelling, are limited in scope: in general, data have been generated in single-center studies or preclinical models. Here, we summarize many of the main findings of the past several decades of research on this topic, and propose areas of focus for future research that will facilitate investigating the microbiome ands its role in disease (Table 1).

The earliest days of microbiota research: germ-free mice and patients

Pioneering studies conducted in the early 1970s demonstrated that mice undergoing allo-HCT in germ-free conditions experienced less GVHD and had improved survival (Table 2)[4–16]. Soon thereafter, this observation led to attempts to reproduce these conditions in patients undergoing HCT through the use of laminar-airflow isolation rooms, "sterile" diets, gut decontamination with oral non-absorbable antibiotics, and skin cleansing (Table 3)[17–29]. Between-study heterogeneity and the lack of reproducible data supporting efficacy for GVHD prevention have limited evidence-based guidance for clinical practice and prophylactic strategies. However, while many of these previous approaches have largely been abandoned, broadly-adopted modern-day recommendations to prevent infectious complications include antimicrobial prophylaxis, sterilized positive air pressure rooms, low-microbial diet, and use of barrier precautions (e.g. gloves, face masks, gowns)[30]. Recently, there has been a resurgence of microbiome research across many disciplines of medicine spurred by advances in high-throughput methodologies for characterizing the microbiome, which extend beyond bacterial culture techniques and virus-specific molecular approaches for detection. Similarly, there has been rapid growth in the microbiome research as it relates to HCT.

Section I: Methods to investigate the microbiome

With the advent of high-throughput molecular methods to study the microbiome, the field has grown significantly in the past decade. Commonly used methods include 16S ribosomal RNA gene sequencing for bacterial taxonomic classification, metabolomics, as well as shotgun metagenomic sequencing and subsequent taxonomic and functional classification of microbial genes; these methods have been reviewed in detail elsewhere (Table 4) [31,32–34].

Each of these methods provide an orthogonal approach to study the microbiome from the perspective of answering important microbiota taxonomic and functional questions including “which microbes are there?”, “what do they make?” , “what genes do they contain?”, and “what is their relative and absolute abundance?”[35]. With the explosion of new molecular and bioinformatic approaches to study the microbiome, we anticipate an ever-growing toolkit to characterize potentially clinically relevant features of the microbiome such as antibiotic resistance, microbial virulence factors, and strain dynamics. Terms commonly used in microbiome studies and their definitions are listed in Table 5[32,36–38,39].

A precedent has recently been set for the generation of multi-faceted data types (ranging from shotgun metagenomic sequencing to transcriptomes and epigenomes to metabolite profiling) that facilitate multi-dimensional and longitudinal characterization of both the host and the microbiome. Specifically, projects of the integrative Human Microbiome Project (iHMP), the US National Institutes of Health Common Fund’s second phase Human Microbiome Project, have collected longitudinal samples from three cohorts of individuals (comprising individuals with pregnancy and pre-term birth, type 2 diabetes, and inflammatory bowel disease)[40,41]. Given these advances in “multi-omic” data collection, we anticipate that the next decade of translational research in the microbiome field as it relates to HCT will extend far beyond simple characterization of community taxonomic structures within the microbiome. For example, advances in immunophenotyping and short-term, *in vitro* propagation of microbial mixtures will identify potential mechanistic relationships between microbes, microbial antigens, and host responses[42,43].

While the advances in phenotyping and genotyping experiments may pave the way for the identification of biomarkers that may be clinically actionable, there are challenges and limitations to their effective, wide-scale application. For example, a specific challenge is the need for rapid turnaround of next-generation sequencing results to be clinically actionable; at present, this is not routinely available due to the need to batch samples to reduce the costs of sequencing. Thus, while next-generation sequencing and metabolomic approaches are the predominant technologies used in the research setting, some intriguing and potentially more easily deployed alternatives for microbiome measurement may be more translatable. Indeed, these technologies do exist and include approaches such as species- or bacterial-group-specific quantitative PCR[33] and microarray approaches[34,44]. Despite the challenges to their clinical use, it is feasible for advancements in microbiota science currently based on next-generation sequencing to be optimized for real-time use both diagnostically and prognostically in the HCT setting.

Open Questions

- How do we design and execute microbiome studies that permit simultaneous characterization of a) microbial characterization beyond taxonomy, b) both host impact on and host response to the microbiome, thus informing a deeper understanding of microbiota function?
- What new technologies (such as single cell sequencing, T-cell receptor sequencing, long-read sequencing and advanced imaging) will emerge as new

ways to measure the microbiome, its structure, function and interactions with the host; how should we apply these approaches in the HCT setting?

- What strategies for multi-center collections of biospecimens and clinical data best support future integrative “multi-omic” approaches to illuminate host-microbe relationships as they pertain to HCT outcomes?

Section II: The Microbiome as Biomarker

Identifying biomarkers with high prognostic and predictive value is crucial for communicating risk to patients and selecting appropriate therapeutic strategies. Thus, it is no surprise that composition of the intestinal microbiota, which is affected by host genetic factors, immunological factors, diet, medications, lifestyle and environmental exposures, has been analyzed as a biomarker for important clinical outcomes after HCT. During transplantation, dramatic shifts in the composition of the intestinal flora are observed[1]. These shifts in species abundance and measures of diversity have been proposed as potential biological markers associated with patient outcomes after transplantation (Table 6)[1,2,3,22,27,45,46,47,48,49,50] Biomarkers such as these may prove useful in the design of clinical trials to identify patients at risk of certain outcomes, as surrogate markers of clinical outcomes, or as early predictive markers of treatment response. If a causal relationship between the microbiota and transplant outcomes can be established, these relationships may inform the development of microbiota-based therapeutic interventions to improve transplant outcomes.

3-indoxyl sulfate as biomarker of intestinal microbiota health

An example of a recently proposed microbiome-derived biomarker in HCT is the small, aromatic tryptophan metabolite 3-indoxyl sulfate[47,51]. Indoxyl sulfate originates from the degradation of dietary protein-derived tryptophan to indole by the tryptophanase of commensal intestinal bacteria. After resorption of indole from the intestine, it is metabolized to indoxyl sulfate in the liver and finally excreted in the urine. Microbiota-derived indole and its derivatives are integral to the maintenance of human microbial communities through bacteriostatic effects on Gram-negative enteric bacteria, antifungal activities that provide colonization resistance to *Candida albicans*, as well as regulation of epithelial function and control of local inflammation through induction of anti-inflammatory cytokines[52,53]. Recent studies have demonstrated that indoxyl sulfate can serve as an important biomarker with lower urine levels being associated with significant and clinically relevant intestinal microbiota disruption in patients undergoing allo-HCT[3,47,54]. In the future, metabolites such as indoxyl sulfate may serve as a urine or serum marker for monitoring microbiota perturbations in patients being treated with antibiotics or in predicting the development of GVHD[47].

Open Questions—

- Can the composition of the intestinal microbiota serve as a biomarker for clinical outcomes after HCT and can it be used to guide interventions?

- Are there specific microbes that are causally associated with positive or negative outcomes of HCT, perhaps through their antigenic properties or through the action of generated metabolites? If so, might there be strain-specific differences in their capacity to induce inflammation or cytotoxic damage?
- How can changes in the microbiome be assayed in real-time to allow for clinical decision-making?

Section III: Interactions Between the Microbiome and the Immune System

The microbiome and development of the immune system

Proper immune reconstitution is central to successful HCT. To better understand immune reconstitution in patients following HCT, it is helpful to turn to the well-studied and analogous process of immune development in neonates. The adaptive immune system and microbiota undergo a process of rapid change and development over the first three years of human life, and these two processes are intimately interconnected[55]. Developmental microbiota perturbations have been associated with short-term immune consequences early in life, and there is a strong suggestion that these early perturbations may have long-term deleterious effects on immune function as well[56,57,58,59,60,61]. As successful immune reconstitution is central to HCT efficacy in the short- and long-term, it is critical to understand exactly how the microbiota impacts that process.

Axenic or “germ-free” animal models have been an essential tool in defining the importance of microbes to immune development[62,63,64]. Studies in these systems have shown that the immune system of a germ-free neonate is under-developed[65]. Most notable are the changes observed in mucosal immunity, particularly in the intestine, with absence of gut-associated lymphoid tissue, including isolated lymphoid follicles, Peyer’s patches, and mesenteric lymph nodes[66]. While many similarities exist between the immunologic development process in infants and HCT recipients, some differences must also be considered. Notably, the adult intestinal microbiome is quite divergent from the infant microbiome, and individuals undergoing HCT have often received antimicrobial and other pharmacological agents that damage the microbiota composition. Thus, while the microbiota likely plays a role in immune reconstitution post-HCT, we must carefully consider both the similarities and differences to the process of infant immune development as we try to understand how the microbiota impacts both immune reconstitution and adverse immunological post-HCT outcomes such as GVHD.

The microbiome and its role in immune reconstitution post HCT

Impaired immune reconstitution after HCT is a significant cause of morbidity and mortality, and has been implicated in increased risk of infections, malignancy relapse, and development of secondary malignancies[67,68,69,70,71,72,73,74,75]. Given recent data on the interactions between the gut microbiome and transplant outcomes discussed above, as well as the immune system[76,77,78], it is reasonable to hypothesize that the microbiome plays a direct role in post-transplant immune recovery. The study of post-transplant immune reconstitution now benefits from a variety of quantitative and qualitative assays, ranging from clinical parameters such as absolute lymphocyte counts (ALC), lymphocyte subsets

(CD4$^+$ and CD8$^+$ T cells, NK cells, B cells) and antibody titers, to more complex functional assays and evaluations of T-cell and B-cell repertoire, to next-generation sequencing approaches to provide information on TCR diversity and specific clonotypes over time[79]. Currently, no integrated datasets comprised of simultaneous host and microbiome measurements are publicly available for analysis. Future prospective studies will need to integrate these areas of research to better define potential interactions between the immune system and the microbiome in HCT, as has been done in the iHMP for other diseases[41,80].

Potential mechanisms of immune modulation by the microbiota that impact GVHD and GVT

The largest proportion of microorganisms in the body exists in the lower intestine, thus the intestine is believed to be the major interface between the microbiome and adaptive immune system. Intestinal homeostasis is a dynamic process that includes maintenance of bowel mucosa integrity and relies heavily on the interactions between immunologic function and the community of organisms that make up the gut microbiota. HCT leads to dysbiosis and disruption of intestinal homeostasis as a result of the conditioning regimen, use of broad-spectrum antibiotics, alterations in nutrition, and donor cell-derived immune reconstitution. There is clinical evidence for the regulatory effect of gut microbiota in the maintenance of intestinal homeostasis mediated primarily through regulatory T cells[81,82,83]. For example, emerging data suggest that alterations in the intestinal microbiota and metabolome are associated with the incidence and severity of acute GVHD (Tables 7a and 7b) [1,3,22,26,27,29,48,49,50,54,76,84,85,86,87,88,89,90,91,92,93,94,95,96]. While the majority of the literature has focused on changes in intestinal microbiota diversity, others have focused on the role of particular organisms, such as *Blautia spp*. in protection from GVHD[48]. Reports of GVHD associated with blooms of eukaryotic viruses, such a picobirnaviruses, have also begun to emerge, suggesting a potential role for the human virome as, at the very least, a marker of this transplant complication[50]. In addition to increasing GVHD risk, disruption in intestinal homeostasis and dysbiosis is associated with increased treatment-related mortality (TRM), and decreased overall survival (OS) [2,3,49].

Commensal bacteria can also play a role in tumor immunosurveillance. Although the precise mechanisms by which intestinal microbes can promote tumor immunity are unknown, one hypothesis invokes antigen mimicry, as microbial proteins can bear close resemblance to tumor associated antigens[97]. An alternative pathway might be through non-specific activation of innate immune cells and pathways. Consistent with the notion of the microbiome influencing anti-tumor immunity, it was recently shown that specific members of the intestinal microbiota are associated with a decreased risk of relapse after allo-HCT[2]. Achieving a comprehensive understanding of the mechanisms driving both mucosal and systemic immune modulation by the gut microbiota may facilitate the simultaneous mitigation of GVHD while maintaining or improving GVT effects.

Whether particular microbiota signatures correspond to a causal or contributing factor to the development of various disease phenotypes remains to be elucidated, as most clinical studies have established only associations, with rare exception[22]. Specifically, analyses of alterations in intestinal microbiota have focused on time-course compositional descriptions

and correlations with clinical and biological outcomes, in particular acute GVHD. Future research will undoubtedly bring greater focus on both intestinal and extra-intestinal microbial alterations and their mechanistic impact on the development and severity of acute and chronic GVHD in addition to other transplant outcomes. The experimental data so far indicate that modification of the gut ecosystem to restore intestinal homeostasis may represent a novel approach to modulate complications of HCT. While attempts at altering therapeutically the established intestinal dysbiosis could potentially improve transplant outcomes, we are in the beginning phases of comprehending the full impact and the mechanistic role of microbiota in HCT, not only for GVHD outcomes but also for tumor relapse, infectious complications, and long-term outcomes after HCT.

Open Questions—

- Can we identify specific associations between the microbial taxa, antigens or metabolites and post-HCT immune recovery?
- Is the pre-treatment microbiome (prior to any treatment for the underlying disorder) prognostic of immunologic and other outcomes post-transplant?
- What is the role of oral and skin microbiomes as well as microbes from other organs on the incidence and severity of acute and chronic GVHD? Is there a specific set of intestinal and extraintestinal (e.g. ocular, skin, vaginal) microbial taxonomic structures over time that correlate with or are causally related to chronic GVHD and late effects of HCT?
- How can we use interventions that modify the microbiome to improve post-HCT outcomes, specifically mediated by immune effects on GVHD and GVT?
- Do specific T-cell responses against bacterial antigens affect donor and recipient T-cell repertoires and therefore play a role in HCT outcomes?
- Do donor lymphocyte infusions, checkpoint inhibitors, CAR T-cell and other T-cell therapies impact the microbiome? If so, does the microbiome in any way mediate clinical outcomes following these therapies?

Section IV: The Microbiome and Its Role in Infection and Idiopathic Post-HCT Disorders

Infection is a major cause of non-relapse morbidity and mortality after HCT, second only to GVHD. Unfortunately, other than administering prophylactic antibiotics or antiviral agents to susceptible patients, the therapeutic approach against these infections is largely reactive. Thus, identifying modifiable host or microbiome features that can be manipulated to prevent infection is a very attractive and promising proposition. The gut microbiota plays a critical role in maintaining colonization resistance against intestinal pathogens, and the mechanisms that underlie this regulation are becoming increasingly well understood. The composition of intestinal microbiota is actively regulated by a number of internal and external factors, ranging from diet to antibiotics, to the elements of the adaptive and innate immune system. An important element of the innate immune system that shapes the microbiota is host-

derived antimicrobial peptides (AMPs). Examples of such AMPs include Paneth cell-derived α-defensins and REG3α, which selectively eliminate non-commensals while preserving commensals, and thus serve as microbiome modulators[98,99]. Intestinal commensal bacteria can stimulate the gut epithelium to produce AMPs that kill pathogenic bacteria[100] and fungi[52]. In GVHD, for example, Paneth cell loss is associated with both reduced secretion of α-defensins and intestinal dysbiosis[85,101,102]. Additionally, disruption of gut microbial communities by antibiotics can increase susceptibility to intestinal pathogens[103,104]. Microbiota disruption that leads to gut microbial monodominance (e.g. a microbiome dominated by Enterobacteriaceae or *Enterococcus spp.*) precedes and significantly increases the risk of bacteremia (with Enterobacteriaceae or *Enterococcus spp.*) in HCT patients[45]. In pre-clinical models, a one- to two-log fold reduction in bacterial[105] or fungal[52] gut colonization levels is sufficient to significantly decrease pathogen dissemination and mortality. Similarly, specific gut commensals can provide resistance to *Clostridium difficile* infection[103]. Thus, efforts targeted at protecting the commensal microbiome may protect against intestinal pathogens and infections.

The role of microbiome-host crosstalk and whether specific molecules or pathways in this crosstalk can be manipulated toward therapeutic benefit remains an active field of investigation. Oral administration of synthetic AMPs may restore gut ecology and shape the host immune system to decrease the risk of infection as well as reducing GVHD, while preserving the graft-versus-leukemia effect. As with nearly all antibacterial agents known to date, resistance to specific AMPs has been described[106]. An alternative strategy that leverages a larger spectrum of AMPs and thus protects against rapid acquisition of resistance might be stimulation of the intrinsic production of AMPs. Although augmenting innate cellular function or mucosal integrity is difficult, it may be possible in the future through modulation of gut microbiota or directly inducing gut mucosal immune effectors to tip the balance back towards gut homeostasis, restore colonization resistance, and reduce the risk of severe infections.

In addition to efforts focused on microbiome modulation to protect against bacterial infections, the importance of microbiota in the control of viral infections and host immune responses has been increasingly recognized. Studies specific to HCT patients or to viruses commonly encountered in HCT (i.e. CMV, EBV, adenovirus, and RSV) are still very limited. The interactions among microbiota, host immune response, and viral infections are complex and multi-directional: for example, the microbiome may influence viral-specific CD8 T cell memory[107], which can modulate clinical symptoms, severity, and clearance of viral infections[108], or in reverse, a viral infection may result in a change in the microbiome through host-immune responses and changes in cytokines, including interferon[109]. Pathogenic or nonpathogenic viruses within the respiratory tract[110], skin, or gut[111] may also interact with the bacterial microbiome in what has been termed “trans-kingdom interactions”[112]. Host immune responses to prophylactic vaccines or anti-viral drugs can also be influenced by the status of the microbiota, and this may impact future decision-making around routine decisions such as the schedule for immunizations post-HCT[113].

A proportion of non-relapse related mortality in HCT patients results from non-GVHD and noninfectious complications for which clinicians are unable to ascribe a clear etiology.

These so-called “idiopathic” disorders may be related to an underlying microbiome dysbiosis or a potential infectious trigger that sparks a self-perpetuating inflammatory cascade (i.e. a “hit and run” phenomenon)[114,115]. The application of next-generation sequencing methods and ultrasensitive molecular methods for both unbiased and candidate-base pathogen detection have illuminated several of these “mystery” cases[114,116],. However, recurrent and abundant candidate pathogens have not yet been identified for highly morbid diseases such as the idiopathic pneumonia syndrome. While the evidence is still preliminary in most cases, it is proposed that the microbiome or novel opportunistic pathobionts may contribute to these disease phenotypes on occasion.

The role of the microbiome in modifying the incidence and clinical outcomes of infection and idiopathic disorders in HCT patients is becoming increasingly recognized. To date, most research has focused on the bacterial contribution to these disorders, but increasingly, there is an appreciation of the contribution of viruses to these disorders and to the delicate balance of the microbiome. Both host and microbial factors participate in a complicated interplay to maintain homeostasis, and we are just now starting to understand the detailed elements in this complicated interaction. Little is known about the fungal contribution to both the healthy and diseased HCT microbiome, although we anticipate this will be an area of active and productive research in the future.

Open Questions

- What is the composition of the human “virome” and “mycobiome” in HCT patients - and how do interactions between viruses, fungi, bacteria and the host impact HCT outcomes?
- Do antimicrobial prophylaxis strategies adversely impact the microbiota and render HCT recipients susceptible to opportunistic infections beyond *C. difficile?*
- Might microbiome-targeted therapeutics, aimed at protecting against loss of diversity in the microbiome, decrease the rate of infectious complications such as enteric Gram-negative and Gram-positive bacteremia believed to originate from the intestinal microbiome?
- Which of the “idiopathic” complications of HCT are related to either infections or microbial dysbiosis? In cases where the offending organism acts through a “hit-and-run” type of mechanism, how might we identify these etiologies using existing technologies and sampling strategies?

Section V: Methods for Microbiota Modification

A clear rationale exists for targeting the microbiome with the eventual intention of both fine-tuning the immune system (balancing GVHD and GVT, for example) and decreasing the risk of downstream infectious complications of HCT. Several interventional studies are ongoing that will alter microbiota by means of diet and prebiotics, antibiotics, probiotics, microbial metabolites, and fecal microbial transplantation (Table 8). Below, we will discuss several clinical microbiome manipulation strategies and the implications of their use in future studies.

Antibiotics

Over the past 10 years, metagenomic and other culture-independent microbiota analyses have demonstrated the important role of the microbiome in health and disease[117]. Patients undergoing HCT represent a natural group for this line of research for the reasons that they are a) uniquely prone to perturbations in the normal microbiome as a result of toxicity from conditioning regimens, impaired diet, and antimicrobial exposure given for treatment and prophylaxis, b) their propensity for infectious and immune-mediated morbidity and mortality, c) their prolonged peri-transplant hospitalization that facilitates convenient sampling, and d) the availability of long-term outcomes that are universally gathered from transplant recipients. Early studies of prophylactic antibiotics in HCT demonstrated reductions in both infections and GVHD after suppression of the microbial flora[26]. Although the use of broad-spectrum antibiotics has led to dramatic improvement in infection-related TRM, antibiotics result in substantial microbiota disruption[54]. Importantly, the type of antibiotic therapy may determine the composition of intestinal microbiota and the extent of microbiome disruption. For example, antibiotics with anaerobic activity are associated with higher rates of GVHD-related mortality[88]. These new insights, which suggest an unfavorable impact of broad-spectrum antibiotics on intestinal microbiota and patient outcomes after HCT, raise the question of how we might preserve the protective effects of “healthy” commensal organisms without compromising treatment efficacy. In addition to the type of antibiotic, timing of treatment also appears to influence microbial diversity and may impact patient outcomes. Patients starting antibiotics before their day of transplantation showed significantly more microbiome disruption and had a higher TRM than those who began antibiotics on or following day 0 or who did not receive antibiotics[54]. Collectively, these studies support an argument for more selective use of broad-spectrum antibiotics along with early de-escalation strategies. Such strategies would preserve the microbiome but still ensure adequate prevention and treatment of bacterial infections. Further, the benefit of gut decontamination and prophylactic antibiotics should be examined through well-designed prospective trials. Definitive support for or against these practices will only come through the conduct of multicenter prospective trials designed to assess the short-term risk of bacterial infections during neutropenia with long-term endpoints (GVHD, immune reconstitution and microbial resistance) that may be affected by disruption of microbiome diversity.

Open Question(s)—

- How do we balance adequate prevention and treatment of bacterial infections with preservation of the microbiome in HCT recipients?
- How can we incorporate microbiota stewardship practices in addition to antibiotic stewardship practices in our care of HCT recipients?

Diet, Prebiotics, Probiotics, and FMT

Ingested food contaminated by microbes has long been recognized as a potential source of bloodstream infection during chemotherapy-induced neutropenia with attendant gastrointestinal mucosal damage. The germ-free “sterile” diet was conceived in the 1960s as a way to reduce ingestion of potentially harmful microbes, but this was not palatable[118]. A

"cooked-food" diet alternative, which eliminated raw foods with high bacterial counts, was shown in a randomized trial to have a similar effect on bacterial stool cultures as the germ-free diet, but it was also limited by patient dissatisfaction[118]. To expand and improve food palatability, Pizzo and colleagues cultured commercially available foods and identified low-microbial foods that were deemed suitable for a neutropenic diet[119]. The composition of neutropenic diets vary from center to center but, in general, consist of cooked and canned food products and exclude raw meat, fresh fruits, juices and vegetables, raw eggs and unpasteurized dairy products[118,120]. Despite limited evidence to support the merits of a neutropenic diet in HCT recipients as illustrated in Table 9[121,122], dietary restriction of fresh fruits and vegetables continues to be standard practice for neutropenic patients in some centers, which likely has an impact on the amount of fiber that is consumed by HCT patients[123]. In the early post-transplant period, nutritional oral intake often declines to the point of necessitating nutritional supplementation. Retrospective comparisons of parenteral and enteral nutrition have suggested a benefit to the enteral route[124,125]. This may be due to enteral nutrition maintaining digestive function and the mucosal barrier, thus preventing bacterial translocation[126]. An ongoing trial is currently evaluating enteral vs. parenteral nutrition[127].

Specific elements of diet, called prebiotics, are particularly influential in the structure and function of the microbiota. The term "prebiotic" is traditionally applied to indigestible carbohydrates that are metabolized by gut bacteria to produce short chain fatty acids (SCFAs); recently the term is being redefined to refer to any substrate that is selectively utilized by host microorganisms and that confers a health benefit[128]. Apart from a single retrospective study, little is known about how prebiotics affect transplant outcomes such as GVHD[129,130]. However, studies in patients with inflammatory bowel disease treated with prebiotics such as inulin and fructo-oligosaccharides (inulin-type fructans or ITF) have demonstrated that these agents increase microbiota diversity and are associated with a corresponding decrease in disease markers and activity[131,132],. Such studies provide a compelling rationale for studies of prebiotics in the HCT setting.

Probiotics are live microorganisms given to improve health and have long been used as part of traditional diets through the ingestion of fermented foods. Encapsulated preparations of one or more isolated live organisms have been used in attempts to treat a wide variety of gastrointestinal illness including infectious diarrhea or gastroenteritis[133], and inflammatory bowel disease[13490]. Some of these studies have shown evidence of efficacy, most likely mediated through direct antimicrobial effects, stimulation of immune responses that lead to up-regulation of anti-inflammatory cytokines and IgA, and promotion of intestinal barrier function[135,136]. To date, probiotics in the HCT setting have been limited to pre-clinical models and small pilot trials (Tables 10a and 10b)[89,90,137–140]. Of course, concern exists for the potential infectious complications associated with administration of live microbial organisms in high dose[141]. Indeed, case reports of bacteremia following ingestion of probiotics suggest the importance of exercising caution and judgment in the use of live bacterial therapies[142]. Further clinical studies are needed to fully determine the safety and efficacy of probiotics in patients undergoing HCT.

Finally, fecal microbiota transplantation (FMT) is yet another intervention that could be employed to preserve or restore the GI microbiota in patients undergoing HCT. Pioneering physicians performed FMT in non-HCT patients with recurrent or refractory *Clostridium difficile* infections (CDI) and demonstrated efficacy in up to 90% of treated patients[143,144]. Literature on FMT in HCT patients is still scant; however, the limited data to date appears encouraging with a total of 25 reported HCT patients having undergone FMT without known complications (Table 11a–c)[145–151,152]. Highlighting the need for a cautious approach in HCT populations, case reports in non-HCT patients with CDI have documented infectious complications following FMT including norovirus infection and sepsis as a result of presumed bacterial translocation[153,154]. Taken together, while limited published experience suggests that FMT can be used in immunocompromised patients with CDI,[155] prospective trials evaluating safety and efficacy in HCT recipients are needed.

Undeniably, the field of FMT is rapidly growing, yet several questions remain including what guidelines the field should adopt for identification of the best FMT donors and appropriate donor stool screening prior to FMT in immunocompromised patients. Autologous FMT donation may have an advantage of simple traceability of the preparation and control of the inoculum during donor procedures, as well as reducing the risk of potential transmission of diseases originating from the microbiota of an external donor. The opportunity to obtain a ‘pre-morbid/baseline’ stool may not always be feasible for autologous FMT and for this reason, several investigators are using 3rd-party FMT obtained from healthy donors[156]. Case reports have explored the role of FMT for the treatment of non-infectious complications. Finally, it is interesting to speculate about the role of the stem cell donor’s microbiota[96,86], or the patient’s cohabitating family members[157] from whom the microbiota may potentially reconstitute after transplant-induced dysbiosis.

Open Questions—

- What is the impact of neutropenic dietary restriction and use of parenteral nutrition on long-term outcomes post-HCT?
- How might the screening protocol for FMT donors to HCT patients differ than standard screening protocols used for less immunocompromised patients?
- Can targeting of specific microbes or microbial pathways result in modification of microbe-disease associations?
- Is there a role for genetically-modified bacteria in the post-HCT setting? How might this tool be safely and effectively leveraged?
- Are there novel bacterial natural products (small molecules, proteins, glycolipids, sugars) that can be investigated for salutary drug-like effects in the HCT patient population?
- Might we create a defined microbial consortium as the next-generation microbial therapeutic to supplant FMT in the HCT recipient population?

- How stable are microbial populations and microbial genomes over time? How does horizontal gene transfer affect the medium- and long-term safety and efficacy of potential novel bacterial therapies?

Section VI: Maximizing the Opportunity for Impact

Identifying associations between the microbiome and clinical phenotypes is critically important. With the advent of technologies such as metagenomic sequencing, metabolomics, and improved tools for studying human immunology, it is becoming increasingly affordable and feasible to perform longitudinal molecular characterization of patients following HCT. As we transition toward an increased reliance on human samples for the generation and querying of biological hypotheses, it is important that the same rigor used in carefully controlled *in vitro* or animal experiments be applied in the clinical setting. This is particularly important in light of the sometimes conflicting results seen between pre-clinical and clinical studies, possibly as a result of microbiota variability between animal strains or differences in practice between centers, but also potentially as a result of variation in sample management. Samples must be collected, stored and processed in a reproducible manner to avoid the unintended introduction of bias in the results[158]. This is of utmost importance when studying low biomass or low microbial burden samples, where the chance introduction of ambient microorganisms through handling or processing may confound the ability to draw robust, reproducible, and generalizable conclusions. Similar to standard sample collection and data generation practices, strict procedures for management of data generated through high-throughput techniques will ensure data quality and accuracy critical to reliable interpretation and analysis. For example, current efforts to understand the microbiome and its impact on transplant outcomes are often limited by incomplete information on antibiotic exposure and diet. The BMT community has a strong track record of collecting detailed clinical data regarding GVHD and immunosuppressive medication administration. This same rigor needs to be applied to infection reporting and antimicrobial exposure in order to draw meaningful conclusions regarding microbiome findings. To this end, the CIBMTR currently has a working group tasked with the development of reporting standards for infectious disease endpoints.

As clinical infectious data collection strategies improve, inclusion of patient-reported outcomes (PROs) should also be prioritized in microbiome-oriented studies. PROs provide greater accuracy of treatment-related symptoms than clinician report[159], and given the role that the microbiome likely plays in mediating symptoms such as diarrhea, constipation, flatus and abdominal discomfort, inclusion of PROs will be critical in studying the impact of microbiome-modifying therapies. Specifically, validated instruments of physical function and health related quality of life are available and include patient-reported measures of gastrointestinal symptoms, with domains of severity, frequency, and interference related to these symptoms contained within the NCI PRO-CTCAE (http://healthcaredelivery.cancer.gov/pro-ctcae). The successful incorporation of PROs will require 1) the development of a scalable infrastructure for participating sites, 2) consensus choice of relevant measures[160], 3) collection time points so that PROs can be appropriately linked to clinical data consistently across research studies and clinical practice, and 4) application of statistically sound approaches to handle missing data is of paramount importance.

It is expected that the collection of high quality high-resolution molecular, clinical and patient-reported symptom data will hasten the identification of potentially relevant and useful molecular biomarkers as well as associations between microbiome alterations or specific microbes and clinically relevant outcomes. We anticipate that “big-data” approaches, including clinical-informatics efforts that extract information from the electronic medical record will be a key part of this effort. As more and more data are collected, it will be increasingly important to leverage machine-learning approaches to data interpretation and analysis, as is already being done in the field of cancer genomics and beyond.

Perhaps most importantly, we must not lose sight of the importance of moving “beyond association” in study design. Strategies to model the hypothesized interactions between the microbiome and host, both *in vitro* and *in vivo* in models ranging from cell lines to small and large animal models, will allow for carefully controlled experiments to be carried out - something that is of course difficult or challenged by ethical considerations in patient studies. Lastly, whenever possible, it is critical that well-designed and thoughtfully targeted interventional studies of microbiome modification be performed in multi-center settings. This will provide the highest level of prospective data to help guide clinical practice, and the multi-center nature of these studies will ensure the highest level of generalizability. When these studies are done, adhering to rigorous standards of both biospecimen collection, clinical data collection, and PRO data collection will allow for the accurate measurement of the consequences of the tested interventions. This will shed light on potential mechanisms of action of these interventions, and will inform the next iteration of interventions (Figure 1).

Open Questions

- How can we facilitate more universal sample collection and support multi-institutional studies of the microbiome to improve the generalizability of findings?
- How do we identify and implement standardized methods for sample and data collection that can be used for multicenter prospective clinical trials that study the microbiome in HCT patients?
- How can we best collect information about infections and antimicrobial medication use in both the clinical trial setting and for registry purposes?
- What are the critical clinical data that need to be collected for meaningful analysis of microbiome studies in HCT?
- How can we hasten the design and execution of microbiome-targeted interventional clinical trials? What funding sources exist to support these critically important efforts, given the relative paucity of classical industry partners in this space?

Section VII: Future Directions

While the importance of the microbiome in immunological development, protection against infections, and patient symptoms has been investigated in the past decades, much remains to

be understood on the importance and relevance of the contribution of the stability, the resilience and the redundancy of the microbial composition after transplantation. The field has come a long way from the earliest days of microbiome research, now nearly a half a century ago. There has been an explosion in the number of high-throughput tools for microbiome measurement and an increasing number of single institution biospecimen collections. These tools and resources have facilitated the testing of only a limited number of translationally important hypotheses. The taxonomic diversity of the microbiome has been shown to be a potential biomarker of HCT outcomes, ranging from overall survival to relapse. These early, single-institution findings are certainly compelling, and warrant further investigation. Interactions between the microbiome and immune system have been described for decades; deep immunophenotyping tools such as T-cell receptor sequencing and high-dimensional mass cytometry are now facilitating investigating the temporal relationships between the microbiome and immune system during immune reconstitution. Early data suggest a potential role for the microbiome in improving post-transplant immune reconstitution and helping to achieve the elusive goal of effective GVT without GVHD. A growing set of tools for microbiome manipulation using diet, prebiotics, probiotics and even FMT are being tested rigorously, both in preclinical models and in humans. It is anticipated that larger randomized multi-institutional studies of these approaches and their efficacy will be initiated. Beyond GVHD, the clear role of the microbiome in mediating risk of infection and perhaps idiopathic disorders also poses an exciting opportunity for investigation, and the potential to improve non-relapse, non-GVHD related morbidity and mortality. To maximize the impact of microbiome-focused investigation, there are many targets that represent low-hanging fruit: improving infection and antibiotic-related data collection, incorporating PROs, and the application of newer methods such as shotgun sequencing, metabolomics, metaproteomics, advanced microscopy and beyond, that will allow us to extend our investigational reach beyond the taxonomic realm. A more functional characterization of how these communities are structured, how they interact and what they do will undoubtedly inform progress in developing precision microbiome diagnostic and therapeutic strategies. Advances in technology have revealed many opportunities to better understand the mechanisms that underlie microbiome-host interactions. For example, recent studies have brought to the fore the role of metabolomes and host genes that are critical[89], yet, there have been so far no corresponding studies on the RNA transcripts (metatranscriptomics) and proteins produced by the microbes in these processes in HCT patients. The abundance and transcript levels of genes encoding microbial resistance to antibiotics, drug metabolism and resistance to host mediated immune responses, for example, could shed light on better exploitation of the microbiome in HCT. Advances in bioengineering have resulted in the ability to generate microbes with specific, salutary effects. For example, oral administration of commensal bacteria genetically engineered to regulate endogenous or recombinant gene expression to alter their metabolic ability could hold great promise for restoring intestinal homeostasis and modulating host immune systems[161]. New technologies are rapidly being developed and applied; thus, in the coming years research will better define the role of microbiome on GVHD, GVT, infectious complications, and transplant outcomes. As this happens, we hope that carefully considered and planned investigations ranging from basic microbiology to immunology to large-scale, randomized-controlled interventional clinical trials will together result in improved outcomes for HCT and related patient populations.

Simultaneously, we anticipate that such efforts will result in an improved understanding of the basic biological underpinnings of microbial bioregulation, microbiome community interactions, and human immunology. Vast opportunities exist for both scientific and translational advances in the realm of microbiome sciences. In order to capitalize quickly on these prospects for maximum impact, we propose ten areas of focus (Table 1) that may have the greatest promise for breakthrough discoveries regarding the dynamic and complex microbiota-host relationship in patients undergoing HCT.

Acknowledgments

Funding

TAM is supported by the American Society of Blood and Marrow Transplantation New Investigator Award. ASB is supported by the Damon Runyon Cancer Research Foundation. The Blood and Marrow Transplant Clinical Trials Network is supported in part by grant #U10HL069294 and #U24HL138660 from the National Heart, Lung, and Blood Institute and the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Other disclosures:

In the prior 2 years, MRMVDB has served as a consultant for Jazz Pharmaceuticals, Novartis, Regeneron Pharmaceuticals, Flagship Ventures, Boehringer Ingelheim, Merck, and Evelo

LSK consults with Bristol Myers Squibb and Kymab, Ltd in addition to receiving research funding from these in addition to Regeneron Pharmaceuticals

MMM received honoraria and research support from MaaT Pharma

RRJ has consulted for Ziopharm Oncology

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Highlights

- Microbiome alterations are associated with infection, GVHD, and other adverse transplant outcomes
- "Multi-omic" technologies may better identify clinically actionable microbiome biomarkers
- Functional microbial characterization may improve diagnostic and therapeutic strategies
- Knowledge gaps persist in chronic GVHD and extra-intestinal microbiome-host interactions
- Multi-institutional studies and data collection expansion will maximize microbiome research impact

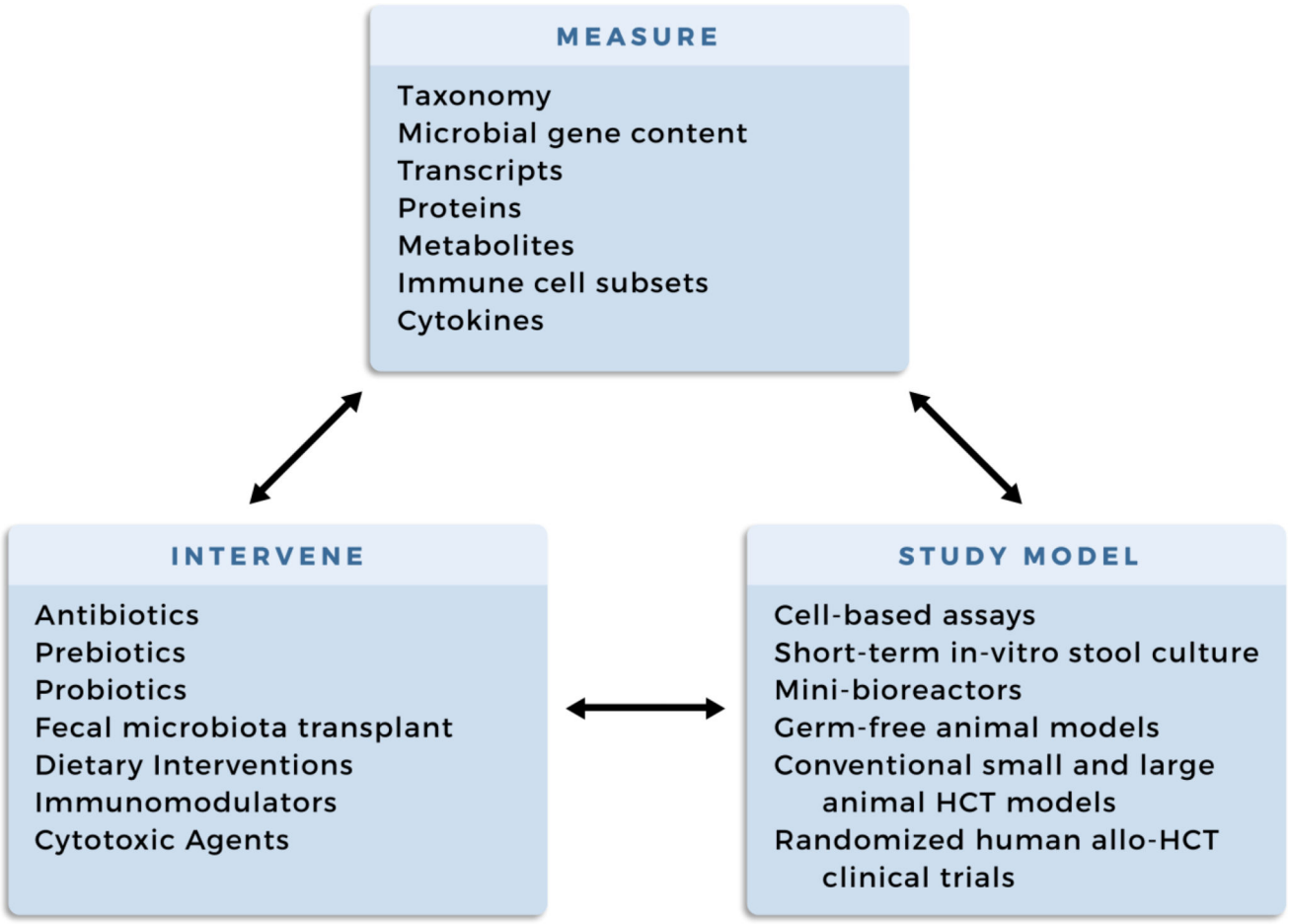

Figure 1.
Structuring the design of future interventions aimed at establishing microbe-host disease causality

Table 1

Proposed next steps for future research investigating the microbiome-host relationship in HCT patients

Primary Research Gaps and Strategies	
1	Multi-institutional prospective studies to improve generalizability of microbiota research findings
2	Multi-center biospecimen and data collection to support collaborative and integrative “multi-omic” approaches to microbiota research
3	Expansion of microbial metagenomic studies in HCT patients beyond bacterial taxonomy
	a. Investigation of the role of the virome and mycobiome in HCT outcomes
	b. Elucidation of microbial genes and metabolic pathways that impact outcomes, in particular the antibiotic resistome
4	Targeting of specific microbes and/or microbial pathways to determine whether modification of the microbiota can impact microbe-disease associations
5	Investigation of the role of extra-intestinal microbial populations (i.e. oral, skin, vaginal, eye) in the development of acute and chronic GVHD
6	Investigation of microbial-host dynamics associated with the development of chronic GVHD and other non-GVHD outcomes
7	Modification of antibiotic strategies that balance preservation and restoration of the microbiome with appropriate infection prevention and treatment
8	Antibiotic-sparing approaches to infection prevention and treatment; steroid-sparing approaches to GVHD prevention and treatment
9	Incorporation of standardized antibiotic and infection-related data into national transplant databases
10	Understanding the potential for using the microbiome as a biomarker for transplant outcomes and as a guide to interventions

Table 2

The early years of microbiota research in germ-free mice

Model system and intervention	Findings	References
Germ-free mice vs. conventional mice	Significantly milder GVHD symptoms and longer survival after MHC*-disparate allo-HCT in germ-free mice	Connell, 1965[4] Jones, 1971[5] Van Bekkum, 1961[6] Van Bekkum,1977[7]
	Intact GVT and reduced GVHD after MHC-disparate allo-HCT in germ-free mice	Pollard, 1973[9] Pollard, 1974[10] Truitt, 1974[11] Truitt, 1976[12]
Germ-free mice vs. conventional mice vs. mice with consortium of colonization resistant intestinal microflora (anaerobes)	Significantly milder GVHD symptoms and longer survival in both germ-free and colonization resistant mice whose microflora was predominantly anaerobic Conventionalization of all mice after 40 days did not did not induce GVHD in gnotobiotic mice that received colonization resistant microflora but did in conventional mice treated with antibiotcs	Van Bekkum, 1974[13]
Antibiotic-treated mice vs. untreated mice	Significantly milder GVHD in xenogeneic rat-to-mouse HCT; GVHD histology present but less inflamed in antibiotic-treated mice compared to conventional mice	Heit, 1973[8]
	Significantly milder GVHD symptoms and longer survival after MHC-disparate allo-HCT in antibiotic-treated mice	Van Bekkum, 1967[14] Heit, 1977[15]
Selective antibiotic decontamination of Enterobacteriaceae vs. conventional mice	Mitigation of delayed-type GVHD by selective decontamination of Enterobacteriaceae was minor and dependent on mouse model	Veenendaal, 1988[16]

*MHC=Major histocompatibility

Table 3

Clinical studies of protective isolation with gut decontamination in HCT patients

Intervention	Control	Outcomes (Intervention v Control)	References
Randomized trials			
Protective isolation (LAF* isolation, skin cleansing, sterile diets) + oral antibiotic decontamination (n=45)	Oral antibiotic decontamination (n=44)	• Decreased risk of infection • Fewer patients with septicemia • Fewer days with septicemia • Longer time to first major infection • Later onset GVHD (only in aplastic anemia patients) • No difference in survival • Of note, adherence to oral antibiotic decontamination was poor	Buckner, 1978[17]
LAF* isolation + oral antibiotic decontamination (n=36)	Conventional rooms +hand-washing and mask precautions (n=31)	• Decreased incidence of infections after engraftment • Later onset acute GVHD • Trend toward decreased incidence of grade II to IV aGVHD; not statistically significant • Increased survival	Navari, 1984[18]
LAF* isolation + oral non-absorbable antibiotics + prophylactic systemic antibiotics (n=54)	LAF* + oral nonabsorbable antibiotics (n=68)	• Fewer episodes of septicemia • No difference in incidence or severity of GVHD • No difference in mortality	Petersen, 1986[19]
Conventional rooms + prophylactic systemic antibiotics (n=45)	Conventional rooms + prophylactic granulocyte infusions (n=67)	• No difference in incidence of septicemia or local major infections • No difference in GVHD incidence • No difference in mortality	Petersen, 1986[20]
LAF* + prophylactic systemic antibiotics (n=49)	Conventional rooms+ prophylactic systemic antibiotics (n=50)	• Decreased septicemia • Decreased major local infections (borderline significance) • No difference in GVHD incidence • No difference in mortality	Petersen, 1987[21]
Conventional rooms + ciprofloxacin + metronidazole gut decontamination (n=68)	Conventional rooms + ciprofloxacin gut decontamination (n=66)	• Decreased incidence of acute GVHD • No difference in chronic GVHD • No difference in OS	Beelen, 1999[22]
Observational trials			
LAF* isolation + oral antibiotic decontamination (n=39)	Conventional rooms + oral antibiotic decontamination (n=91)	• Decreased incidence of acute GVHD	Storb, 1983[23]

Intervention	Control	Outcomes (Intervention v Control)	References
		• Increased long-term survival	
LAF* isolation +oral and topical antibiotic decontamination (n=26)	Conventional rooms + barrier nursing +oral and topical antibiotic decontamination (n=22)	• No difference in GVHD incidence • No difference in mortality	Mahmoud, 1984[24]
Strict reverse isolation** + oral non-absorbable antibiotics, complete gut decontamination (n=26)	Barrier nursing + oral non-absorbable antibiotics, selective gut decontamination (n=15)	• Fewer days of fever and fewer infections • Trend towards decreased GVHD although not statistically significant	Schmeiser, 1988[25]
LAF* + complete antibiotic oral decontamination (n=44)	LAF* + selective decontamination (n=21)	• Fewer infections • Decreased acute and chronic GVHD incidence • Lower combined TRM or chronic GVHD incidence	Vossen, 1990[26]
Sustained growth suppression of anaerobic bacteria with oral nonabsorbable and systemic antibiotics (n=41)	Incomplete growth suppression of anaerobic bacteria despite oral nonabsorbable and systemic antibiotics (n=153)	• Decreased incidence of acute GVHD	Beelen, 1992[27]
Protective isolation with either LAF* or HEPA filters (n=423 8)	Conventional isolation (n=827)	• No difference in acute or chronic GVHD incidence • Decreased TRM and overall mortality in the first 100 days post-transplant	Passweg, 1998[28]
Protective isolation + successful gut decontamination with oral nonabsorbable and systemic antibiotics (n=57)	Protective isolation - successful gut decontamination despite oral nonabsorbable and systemic antibiotic (n=55)	• Decreased infectious risk • Decreased incidence of acute GVHD	Vossen, 2014[29]

* LAF= Laminar air flow

** Reverse isolation was achieved in sterile plastic isolators

Table 4

Sequencing technologies used in microbiome research

Method	Definition	References
Metagenomics	The study of genes and non-coding genetic information in a mixed population of organisms in order to infer functional potential and taxonomic structure of the population and its individual organisms	Marchesi, 2015[32]
16S ribosomal RNA/DNA sequencing	PCR amplification of bacterial RNA/DNA from the variable regions of the 16S ribosomal RNA gene for taxonomic profiling (the region selected is usually determined by the niche that is being investigated; e.g. V4 for stool, V1-3 for skin)	Weisburg, 1991[33]
Shotgun next-generation metagenomic sequencing	High-throughput DNA sequence generation and analysis from any organism or group of organisms via fragmentation, tagging, amplification, and massively parallel or deep sequencing. Allows for taxonomic identification in addition to generating information about gene presence, genetic bioregulation and potential metabolic pathways	Loman, 2012[34]
Metatranscriptomics	High-throughput RNA sequence generation and analysis from any organism or group of organisms via reverse transcription, tagging, amplification, and massively parallel or deep sequencing. Provides a snapshot of which genes are being transcribed	Marchesi, 2015[32]
Metabolomics	Characterization of the collection of metabolites produced by an organism or a single tissue. The term has been used to described characterization of the collection of metabolites produced by a collection of organisms (i.e. the microbiota), although some prefer the term “metabonomics” for that definition	Marchesi, 2015[32]
Metaproteomics	Characterization of all proteins within a clinical or environmental sample	Marchesi, 2015[32]

Table 5

Definition of terms

Term	Definition	References
Diversity	Measurement of the number of different types (taxa) of organisms and their abundance. Alpha-diversity and beta-diversity refer to diversity within and between samples, respectively	Lozupone, 2012[36]
Dysbiosis	Perturbation of the taxonomic structure and function of the microbiota from the healthy state. This can be associated with the development of disease	Petersen, 2014[37]
Germ-free mice	Mice raised in sterile conditions and free from colonization by all microorganisms. Also referred to as “axenic” mice	Giraud, 2008[38]
Fecal microbiota transplantation	The transfer of stool from a donor to a recipient via either endoscopy, nasogastric/duodenal tube, capsules, or enema for the purpose of altering the intestinal microbiota of the recipient and restoring health. Stool is obtained from healthy related or unrelated donors, and less commonly from the intended recipient	Borody, 2013[39]
Microbiota	The entirety of microorganisms (bacteria, archaea, viruses, fungi, and other eukaryotes) within a specific habitat	Marchesi, 2015[32]
Microbiome	Includes the biotic (microorganisms and their genomes) and abiotic (environmental) factors present within a particular habitat. This definition is modeled after the meaning of the term “biome”. Many in the field use the term microbiome to refer to the collection of genes and genomes within a particular habitat, although this definition is redundant with “metagenome”	Marchesi, 2015[32]

Table 6

Clinical studies examining the microbiota as a biomarker for HCT outcomes*

Microbiota Feature	Association	Sample size	References
Sustained decontamination of gut anaerobes	Lower risk of GVHD	194	Beelen, 1992[27]
Decontamination of gut anaerobes	Lower risk of GVHD	134	Beelen, 1999[22]
Intestinal monodomination by *Enterococcus* and Proteobacteria	Higher risk of bacteremia and intestinal GVHD	94	Taur, 2012[45]
Intestinal monodomination, especially by *Enterococcus*	Higher risk of bacteremia and intestinal GVHD	31	Holler, 2014[3]
Decreased duodenal Paneth cell counts at GVHD	Higher GI GVHD severity, lower GVHD treatment response, and higher NRM**	142	Levine, 2013[46]
Low intestinal microbiota diversity	Lower OS, higher TRM	80	Taur, 2014[1]
Lower urinary 3-indoxyl sulfate	Higher intestinal microbiota dysbiosis, higher risk of GVHD	31	Holler, 2014[3]
Lower urinary 3-indoxyl sulfate	Higher intestinal microbiota dysbiosis, higher TRM, lower OS	131	Weber, 2015[47]
Higher fecal *Blautia* abundance	Lower GVHD-related mortality, higher OS	115	Jenq, 2015[48]
Higher abundance or presence of a cluster of bacteria including *Eubacterium limosum* in fecal microbiota	Lower risk of relapse or progression of disease, higher OS	541	Peled, 2017[2]
Higher gradient of positively to negatively correlated organisms at neutrophil recovery	Higher risk of severe acute GVHD	66	Golob, 2017[49]
Picobirnivirus presence	Severe GI GVHD	44	Legoff, 2017[50]

*All studies are observational except Beelen, 1999

**NRM=non-relapse mortality

Table 7

a. Studies of microbiota changes associated with GVHD in murine models		
Intervention/comparison	**Outcome (Intervention vs. control)**	**References**
Reduced-intensity allogeneic vs. syngeneic HCT	• GVHD accompanied by higher Enterobacteriacea, *Bacteroides* and *Enterococcus spp.*, and lower *Lactobacilli, Clostridia, Bifidobacterium*, and *Bacillus spp.* • Higher *Escherichia coli* associated with GVHD severity and reduced survival • Treatment with ciprofloxacin did not affect severity of GI GVHD	Heimesaat, 2010[84]
MHC-disparate and MHC-matched/ minor antigen-mismatched allo-HCT +/ − donor T-cells	• GVHD associated with: – Loss of intestinal Paneth cells – Reduction in α-defensin expression – Lower levels of Firmicutes and Bacteroidetes – Intestinal *Escherichia coli* expansion and dominance – Lower microbiota diversity • Oral administration of the antibiotic Polymyxin B, active against *E. coli:* – Decreased GVHD severity – Decreased GVHD-related mortality	Eriguchi, 2012[85]
MHC-disparate allo-HCT +/− donor T-cells	• GHVD associated with: – Loss of intestinal microbiota diversity – Higher *Lactobacillus* spp. and Enterobacteriales – Lower Clostridiales • Administration of ampicillin before HCT: – Increased GVHD severity and lethality – Higher *Enterococcus spp.* and Enterobacteriaceae – Lower *Blautia spp.* abundance • Administration of *Lactobacillus johnsonii* prevented ampicillin-induced effects	Jenq, 2012[76]
MHC-disparate allo-HCT using T-cells from specific pathogen-free vs. germ-free donor	• T-cell donor microbiota presence or absence did not alter: – T-cell differentiation and proliferation – GVHD severity – GVHD-related mortality	Tawara, 2013[86]
MHC-disparate allo-HCT +/− intestinal helminth infection	• Infection with murine nematode *Heligmosomoides polygyrus:* – Lower GVHD severity – Preserved GVT effect – Increased Treg abundance and improved immune regulation – Increased survival of GVHD mice	Li, 2015[87]

a. Studies of microbiota changes associated with GVHD in murine models

Intervention/comparison	Outcome (Intervention vs. control)	References
	• Protective effects of helminthic infection dependent on TGF-[2]	
MHC-matched/minor antigen-mismatched allo-HCT with anti-anaerobic antibiotics (imipenem-cilastin or piperacillin-tazobactam) vs. antibiotics lacking in anti-anaerobic activity (aztreonam)	• Treatment with anti-anaerobic antibiotics associated with: – Higher mortality – Higher severity of GI GVHD – Increased GI inflammatory infiltration – Higher levels of IL-23 (mediator of GVHD) • Greater abundance of mucin-degrading *Akkermansia*	Shono, 2016[88]
MHC-disparate vs. syngeneic allo-HCT **1** +/− intragastric lavage of 17 butyrate-producing strains of *Clostridia spp.* **2** Anti-anaerobic antibiotics +/− *Clostridia spp.*	• Gavage with *Clostridia spp.* resulted in lower GVHD severity and higher survival • Anti-anaerobic antibiotics followed by gavage by *Clostridia spp.* replicated these findings	Mathewson, 2016[89]
MHC-disparate allo-HCT + levofloxacin **1** +/− clindamycin **2** Clindamycin +/− anti-inflammatory *Clostridia spp.* (AIC)	• Treatment with clindamycin decreased survival • Clindamycin treatment + AIC increased survival	Simms-Waldrip, 2017[90]

b. Microbiota changes associated with GVHD in clinical studies

Intervention or observational group	Control	Outcomes (Intervention v Control)	References
Randomized trials			
Ciprofloxacin with metronidazole prophylaxis (n=68)	Ciprofloxacin prophylaxis (n=66)	• Lower incidence and severity of acute GVHD • No difference in chronic GVHD or OS	Beelen, 1999[22]
Observational trials			
Complete GI decontamination (n=40)	Selective GI decontamination (n=18)	• Lower incidence of acute and chronic GVHD • Lower rate of infection • Lower combined TRM and chronic GVHD	Vossen, 1990[26]
Sustained suppression of anaerobic intestinal flora (n=41)	Incomplete suppression of anaerobic intestinal flora (n=153)	• Lower incidence of acute GVHD	Beelen, 1992[27]
Acute GVHD (n=8)	No GVHD (n=10)	• Lower intestinal microbiota diversity • Higher Lactobacillales • Lower Clostridiales	Jenq, 2012[76]
Successful GI decontamination (n=57)	Unsuccessful GI decontamination (n=55)	• Lower incidence of acute GVHD	Vossen, 2014[29]

b. Microbiota changes associated with GVHD in clinical studies			
Intervention or observational group	**Control**	**Outcomes (Intervention v Control)**	**References**
		• Lower infectious risk	
Lowest GI microbial diversity at engraftment (n=34)	Intermediate or high GI microbial diversity at engraftment (n=20 intermediate, n=26 high)	• Lower OS • Higher TRM, specifically mortality related to GVHD or infection	Taur, 2014[1]
Colonized with *Candida* in the intestine (n=54)	Not colonized with *Candida* in the intestine (n=99)	• Higher incidence of acute GVHD	van der Velden, 2013[91]
Acute GI GVHD (n=8)	No GI GVHD (n=23)	• On the day of transplant: – Higher *Enterococcus spp.* – Lower *Clostridia spp.* and *Eubacterium rectale*	Holler, 2014[3]
Acute GVHD (n=5)	No GVHD (n=5)	• Prior to transplant: – Lower diversity – Lower *Bacteroides* and *Parabacteroides spp.* – Lower proprionate and SCFAs • Between day 0 and day+35: – Higher *Enterococcus spp.* – Lower *Faecalibacterium spp.* • Over all timepoints: – Lower Bacteroidetes	Biagi, 2015[92]
Lower microbial diversity (n=32), lower *Blautia* abundance (n=58)	Higher microbial diversity (n=32), higher Blautia abundance (n=57)	• Lower microbiota diversity and lower abundance *of Blautia spp.* associated with higher GVHD-related mortality • Higher *Blautia* abundance associated with higher OS	Jenq, 2015[48]
Colonized with antibiotic resistant bacteria (ARB) pre-transplant (n=33)	Non-ARB colonized (n=74)	• Higher incidence of acute GVHD and acute GI GVHD • Higher rate of bacteremia, increased infection-related mortality • Higher NRM • Lower OS	Bilinksi, 2016[93]
Treatment of febrile neutropenia with antibiotics effective against anaerobic bacteria:	Treatment with antibiotics less effective or ineffective against anaerobic bacteria: • Cefepime (n=152) • Aztreonam (n=64)	• Higher risk of 5-year GVHD-related mortality • No difference in OS	Shono, 2016[88]

b. Microbiota changes associated with GVHD in clinical studies			
Intervention or observational group	**Control**	**Outcomes (Intervention v Control)**	**References**
• Imipenem-cilastin (n=148) or • Piperacillin-tazobactam (n=300)			
Acute GVHD (n=6)	No GVHD (n=9)	• GVHD associated with: – Higher cumulative antibiotic exposure and anti-anaerobic antibiotic exposure, specifically clindamycin – Higher Enterobacteriacea, *Enterococcus spp.*, and Neisseriaceae – Lower anti-inflammatory *Clostridia*, Bacteroidetes, and Actinobacteria – Depletion *of Ruminococcus* and *Blautia spp.*	Simms-Waldrip, 2017[90]
Pre-transplant antibiotic prophylaxis or treatment (n=239)	No pre-transplant antibiotics (n=261)	• Higher incidence and severity of acute GVHD and GI GVHD • Lower median and 10 year OS	Routy, 2017[94]
Early pre-transplant antibiotics (n=236)	Late post-transplant antibiotics (n=297) or no antibiotics (n=88)	• Lower urinary indoxyl sulfate • Lower Clostridiales • Higher acute and/or chronic GVHD-related mortality • Higher TRM – Higher TRM with ciprofloxacin/ metronidazole vs. rifaximin • Lower OS	Weber, 2017[54]
Pre-conditioning low microbial diversity (n=18)	Pre-conditioning intermediate (n=48) and high diversity (n=41)	• Before conditioning: – No difference in incidence of acute GVHD or GI GVHD – Higher Firmicutes, and a non significant trend toward lower Bacteroidetes in those who later developed aGVHD – No difference in OS, relapse, or NRM	Doki, 2017[95]
Severe acute GI GVHD (n=14)	Non-severe acute GI GVHD or no GVHD (n=52)	• At the time of engraftment:	Golob, 2017[49]

b. Microbiota changes associated with GVHD in clinical studies			
Intervention or observational group	**Control**	**Outcomes (Intervention v Control)**	**References**
		– Lower intestinal microbiota diversity – Higher oral Actinobacteria and Firmicutes – Lower Lachnospiraceae – Higher gradient of positively to negatively correlated organisms	
Acute GI GVHD (n=26)	No GI GVHD (n=18)	• Longitudinal microbiome sampling: – Picobirnaviruses predictive of severe enteric GVHD occurrence – Higher picobirnaviruses before or within a week after transplant – Increased rate of detection and number of sequences of persistent DNA viruses over time – No difference in overall richness – Reduced microbial phage richness over time	Legoff 2017[50]
Acute GVHD (n=34) No GVHD (n=23)	HLA-matched sibling donors (n=22)	• Before conditioning: – Lower recipient intestinal microbiota diversity compared to HLA-matched sibling donors – High donor intestinal microbiota diversity is associated with lower aGVHD incidence in recipients – Low pre-conditioning intestinal microbiota diversity (in recipients) was not associated with higher risk of aGVHD – Lower recipient diversity associated with lower OS	Liu, 2017[96]

Table 8

Clinical trials with microbiota-based interventions in HCT patients

Name	Comparison	Outcomes	Study type	Clinical Trials.gov ID	Status
Dietary Interventions					
Comparing Two Diets in Patients Undergoing HSCT or Remission Induction Chemo for Acute Leukemia and MDS	Standard hospital neutropenic diet vs. diet inclusive of fresh fruits and vegetables	Incidence of major infections	Randomized open-label	NCT03016130	Recruiting
Gluten Free Diet in Preventing GVHD in Patients Undergoing HCT	Gluten-free diet	GVHD	Single-arm	NCT03102060	Recruiting
Randomized, Prospective, Multicenter Study to Compare Enteral Nutrition to Parenteral Nutrition as Feeding Support in Patients With Hematologic Malignancies Undergoing Allo-HCT	Enteral vs. intravenous nutrition	Mortality	Randomized open-label	NCT01955772	Recruiting
Donor Human Milk in Young Children Receiving Bone Marrow Transplantation	Enteral donor breastmilk vs. standard diet	Percentage of stool Lactobacillales	Randomized open-label	NCT02470104	Active, not recruiting
Prebiotic and Probiotic Interventions					
Modification of the Intestinal Microbiome by Diet Intervention to Mitigate Acute GVHD	Bob's Red Mill potato-starch-based prebiotic vs. standard diet	GVHD	Randomized open-label	NCT02763033	Not yet open
Fructo-oligosaccharides in Treating Patients with Blood Cancer Undergoing HCT	FOS	Prebiotic tolerability	Single-arm	NCT02805075	Recruitment completed
Lactobacillus rhamnosus GG in Reducing Incidence of GVHD in Patients Who Have Undergone HCT	Lactobacillus rhamnosus GG supplementation vs. control	GVHD	Randomized open-label	NCT02144701	Active, not recruiting
Fecal Microbiota Transplantation					
Auto-FMT for Prophylaxis of CDI in Recipients of Allo-HCT	Auto-FMT vs. control	CDI	Randomized open-label	NCT02269150	Recruiting
Fecal Transplant for Steroid-Resistant and Steroid-Dependent Gut Acute GVHD	Fecal microbiota transplant	Safety, GVHD	Single-arm	NCT03214289	Recruiting
Antibiotic Interventions					
Choosing the Best Antibiotic to Protect Friendly Gut Bacteria During the Course of HCT	Piperacillin-tazobactam vs. Cefepime during febrile neutropenia	Change in *Clostridial* abundance	Randomized open-label	NCT03078010	Recruiting
Gut Decontamination in Pediatric Allo-HCT	Oral Vancomycin-Polymixin B vs. standard of care	Intestinal microbiota diversity	Randomized open-label	NCT02641236	Recruiting

Table 9

Clinical trials investigating the efficacy of a neutropenic diet in HCT patients

Intervention	Control	Outcomes (Intervention v Control)	References
Randomized controlled, prospective trial			
Unrestricted diet (n=21)	Neutropenic diet (n=25)	• No difference in infectious outcomes • No difference in nutritional status	Lassiter, 2015[121]
Retrospective observational study			
General hospital diet (n=363)	Neutropenic diet (n=363)	• Fewer microbiologically confirmed infections in those receiving a general diet • No difference in the incidence of microbiologically confirmed infections during neutropenia • Higher rate of infections after resolution of neutropenia in those receiving a neutropenic diet	Trifilio, 2012[122]

Table 10

a. Probiotics and the microbiome in murine models of HCT

Treatment	Control	Outcomes	References
Lactobacillus rhamnosus GG	Ciprofloxacin	• Lower mortality • Lower GVHD incidence	Gerbitz, 2004[137]
17 butyrate-producing *Clostridia spp.* strains	Phosphate-buffered saline	• Lower GVHD severity	Mathewson, 2016[89]
Anti-inflammatory *Clostridia spp.* (AIC)	Phosphate-buffered saline	• Higher survival	Simms-Waldrip, 2017[90]

b. Probiotics and the microbiome in clinical studies of HCT patients

Trial design	Probiotic, dose	Outcomes	References
Observational	Self-reported yogurt intake, average of 150g/day (n=41)	• Higher yogurt intake associated with more rapid neutrophil engraftment	Tavil, 2012[138]
Single-arm	*Lactobacillus plantarum*, 1×10^8 cfu/kg/day (n=30)	• 97% of the children received at least half of the probiotic doses • No incidence of *Lactobacillus* bacteremia reported	Ladas, 2016[139]
Randomized	*Lactobacillus rhamnosus* GG, 1×10^{10}/day (probiotic group, n=20; control group, n=11)	• No difference in gut microbiota diversity • No difference in GVHD incidence	Gorshein, 2017[140]

Table 11

a. Fecal microbiota transplantation in auto- and allo-HCT patients with recurrent CDI						
Patient population	**Total patients**	**Route**	**Donor type**	**Outcomes**	**Adverse events**	**References**
21-year-old female; allo-HCT	1	NG tube	Spouse	Resolution of CDI	None	Neemann, 2012[145]
60-year-old female; allo-HCT	1	Push enteroscopy	Anonymous, 2 donors	Resolution of CDI	None	de Castro, 2015[146]
64-year-old male; auto-HCT	1	Enema	Anonymous, 2 sequential FMTs from different donors	First FMT resulted in resolution of CDI. Recurrence in 6 months. Second FMT performed at 6 months resulted in resolution of CDI durable for at least 7 months	None	Mittal, 2015[147]
Allo-HCT patients	7	NJ* tube or colonoscopy	Anonymous	Resolution of CDI in 6 patients; one patient recurred at day +156 post- FMT after receiving antibiotics. Repeat FMT with resolution of symptoms lasting >4 months	None	Webb, 2016[148]
Auto- and allo-HCT patients	8	Oral capsules	Anonymous	No recurrence of disease in 7 patients; one had recurrence of CDI day +179 after FMT	None	Moss, 2017[149]

b. Commercially available agents in Study Pharmacopeia						
Patient population	**Total patients**	**Route**	**Donor type**	**Outcomes**	**Adverse events**	**References**
Steroid-resistant GI GVHD	4	ND* tube	Related	Resolution of diarrhea in 3 patients, partial resolution in 1 patient	None	Kakihana, 2016[150]
Steroid-refractory GI GVHD	3	Colonoscopy	Related or anonymous	Resolution of diarrhea in 2 patients, improvement in GI GVHD in 3rd patient	None	Spindelboeck, 2017[151]

c. Fecal microbiota transplantation in allo-HCT patients with antibiotic-resistant bacteria (ARB)						
Patient population	**Total patients**	**Route**	**Donor type**	**Outcomes**	**Adverse events**	**References**
Hematologic disorders + ARB* colonized	20 (8 HCT)	ND* tube	Anonymous	• 75% of patients (60% of FMTs) achieved complete ARB* decolonization at 1 month • 93% of FMTs achieved complete ARB* decolonization at 6 months	• Vomiting (n=1) • Transient, grade 1 diarrhea within 3 days (n=25) • Abdominal pain (n=2) • Ileus (n=2)	Bilinski, 2017[152]

*NJ: nasojejunal, ND: nasoduodenal,

*ARB: antibiotic-resistant bacteria