Published in final edited form as:

Curr Opin Hematol. 2020 November ; 27(6): 360–367. doi:10.1097/MOH.0000000000000616.

Update in clinical and mouse microbiota research in allogeneic hematopoietic cell transplantation

Sarah Lindner, M.D.¹, Jonathan U. Peled, M.D., Ph.D.^{2,3}

¹Department of Immunology, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA

²Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

³Weill Cornell Medical College, New York, NY, USA

Abstract

Purpose of review: The intestinal microbiota plays a critical role in intestinal homeostasis and immune regulation and has been recognized as a predictor of clinical outcome in patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT) and specifically a determinant of the severity of graft-versus-host disease (GVHD) in mouse models. As GVHD is the most important cause of non-relapse mortality (NRM) after allo-HCT, understanding the mechanisms by which modifying the microbiota may prevent or decrease the severity of GVHD would represent an important advance.

Recent findings: Microbiota injury was observed globally and higher diversity at periengraftment was associated with lower mortality. Lactose is a dietary factor that promotes post-allo-HCT Enterococcus expansion, which is itself associated with mortality from GVHD in patients and exacerbates GVHD in mice. Bacterial and fungal bloodstream infections are preceded by intestinal colonization with a corresponding organism, supporting the gut as a source for many bloodstream infections. Metabolomic profiling studies showed that GVHD is associated with changes in fecal and plasma microbiota-derived molecules.

Summary: In this review, we highlight some of the most recent and important findings in clinical and mouse microbiota research as it relates to allo-HCT. Many of these are already being translated into clinical trials that have the potential to change the future practice in the care of patients.

Keywords

Allogeneic hematopoietic cell transplantation; microbiota; clinical outcome; preclinical studies

Corresponding author: Jonathan U. Peled, M.D., Ph.D., 1275 York Ave, New York, NY 10065, peledj@mskcc.org, Phone: 646-888-2105, Fax: 646-422-0452.

Potential Conflicts of interest:

J.U.P. reports research funding, intellectual property fees, and travel reimbursement from Seres Therapeutics and consulting fees from DaVolterra. S.L. has received travel support from Celgene, Sanofi, and Neovii.

Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a curative treatment for hematologic malignancies in which patients receive a conditioning regimen followed by infusion of hematopoietic cells from a donor. Overall mortality after allo-HCT is in the order of 50% and is largely attributable to relapse of the underlying disease, graft-versus-host disease (GVHD) and infections. Allogeneic donor T and NK cells can mediate protective graft-versus-tumor (GVT) activity, but alloreactive donor T cells can also recognize normal host tissues resulting in GVHD. GVHD is initiated by conditioning-regimen-induced intestinal inflammation and gut-barrier defects that allow transfer of bacterial molecules across the damaged gut epithelium (1). While the gut microbiota is well recognized as the source of these bacterial products, a hypothesis that underpins much work in the field is that the microbiota also modulates additional steps in the pathophysiology of GVHD, perhaps including priming and differentiation of donor T cells or subsequent innate immune inflammatory mediators. Here we focus on the most recent clinical and mouse studies in the context of microbiome and allo-HCT. Several authors have written excellent comprehensive reviews on the subject (2–8).

Main

Microbiota diversity and GVHD-related mortality

In patients undergoing allo-HCT several factors such as diet, antibiotics, conditioning regimen, mucositis, GVHD and infections are hypothesized to influence microbiota composition (6). One key measure of microbiota disruption, α -diversity, reflects the number of unique bacterial taxa in a fecal sample as well as their relative abundances. The first multicenter study profiled 8,767 stool samples from 1,362 adult allo-HCT recipients by means of 16S rRNA gene sequencing and correlated features of the intestinal microbiota with clinical outcomes retrospectively (9). The key observations reported by this international collaborative study (coordinated at our center) were: 1) Microbiota injury which was characterized by loss of diversity and domination by single taxa (most commonly the genus *Enterococcus*) occurred across four transplant centers on three continents; 2) above-median diversity at periengraftment (day 7 to 21), was associated with lower risk of death, mostly related to GVHD in a subgroup analysis and 3) Baseline fecal samples (day –30 to –6) already showed patterns of microbiota injury compared to healthy volunteers and below-median diversity was predictive of poor survival – even when this was only present prior to transplant. These observations seemed to occur independently of (a) differences in clinical practices between transplant centers and (b) geographic variations in the microbiota composition and microbiota-targeted therapies in transplant patients may have broad applicability and were concordant with other recent independent analysis (10).

We also derived a risk-score predictive of mortality based on the abundances of specific bacterial taxa (9), suggesting that in addition to diversity specific bacterial abundances may be predictive of clinical outcome after allo-HCT. A separate single-center study of 141 allograft recipients derived a risk score comprised of day 15 fecal microbiota diversity and abundances of four bacterial families to predict grade II-IV aGVHD (11). In another report, the same group also used linear regression to derive a gut microbiome score that was

predictive of grade II-IV aGVHD in both a discovery and a validation set (12). The strongest predictor within this score, which was based on 20 gut microbiota features measured at day 15, was the abundance of Lachnospiraceae. This observation is strikingly consistent with the observation that Lachnospiraceae are associated with less GVHD (13) and with our previous report that Blautia, a prominent genus within family Lachnospiraceae was predictive of less GVHD-related mortality (14). Moreover, as an indirect measure of gut Lachnospiraceae abundance, urine concentrations of a metabolite produced by this group, 3-indoxyl-sulfate, were previously associated with less transplant-related mortality (15).

These risk scores are best considered as proof of principle that fecal microbiota composition carries some useful predictive information about patient outcome, and specific features that make key contributions to the risk scores can inform hypotheses to test in preclinical models.

Enterococcus domination

A high incidence of fecal community domination by Enterococcus in patients undergoing allo-HCT was previously observed in several studies (9, 16–18). Profiling of fecal samples of 1325 adult allo-HCT patients showed intestinal Enterococcus dominated of up to 65% allo-HCT patients early after transplant and was associated with higher risk of lethal aGVHD (19). Higher relative abundance of Enterococcus in the month following allo-HCT was independently reported to be associated with reduced survival in a cohort of 38 adult patients (20). While in patients, antibiotics are understood to be a major driver of microbiota injuries, including and Enterococcal expansion (17, 18, 21), we also observed an expansion of Enterococcus approximately 1 week after HCT in mouse models of GVHD without any antibiotic treatment (19). Not only did Enterococci expand, treatment of germ-free mice with Enterococcus caused an aggravation of GVHD and accelerated death. Using metagenomic shotgun sequencing, an enrichment of genes encoding enzymes involved in lactose utilization in the Enterococcus-dominated, posttransplant microbiota, which called attention to the possibility that lactose could be a sugar source for these bacteria. A lactose-free diet attenuated the Enterococcus bloom and GVHD severity in mice. This finding is an example of the potential for microbe-targeted dietary interventions to prevent aGVHD.

Interestingly, a recent study revealed, by using whole-genome sequencing of Enterococcus isolates from serial fecal samples, that Enterococcus domination is not a monotonous state but rather one of complex clonal dynamics (22). In line with this, Zlitni *et al.* observed that an apparently stable microbiota composition as assessed by 16S rRNA amplicon sequencing in an allo-HCT recipient actually harbored strain-level differences revealed by an innovative computational analysis of metagenomic DNA and RNA sequences (23). Importantly, both of these studies observed changes in antibiotic-resistance genes during the time courses that were examined.

Another important point to consider regarding Enterococci is that not all members of this genus are necessarily detrimental. Rashidi *et al.* have published a series of studies focusing on *E. gallinarum* and *E. casseliflavus* (24, 25) in which surprising associations with improved survival were observed for these species. In concordance with these findings, in our risk-score for all-cause mortality (9), while Enterococcus as a genus was associated with higher risk of mortality, *E. gallinarum* was actually associated with longer survival.

A lantibiotic protein produced by a strain of *Blautia producta* has recently been demonstrated to attenuate the growth of vancomycin-resistant Enterococci (VRE) (26). Interestingly, this strain belongs to the genus that Jenq *et al.* (14) previously associated with lower GVHD-related mortality and to the family Lachnospiraceae discussed above. It is unknown whether *Blautia* (a) has immunosuppressive properties and directly suppresses GVHD, (b) whether *Blautia* works to suppress GVHD by attenuating Enterococcus, or (c) whether *Blautia* exerts no effect but is simply a reproducibly observed biomarker of microbiota and general gastrointestinal health in allo-HCT patients.

Microbiota and infections

Allo-HCT patients are at high risk for blood stream infections (BSI), especially during the post-transplant neutropenic period, when mucosal injury is at its most severe and domination of the intestinal microbiota with particular pathogenic genera occurs (17). Exploring the longstanding hypothesis that intestinal bacteria are the source of many BSI following stem cell transplantation, Tamburini *et al.* identified that patients with culture-confirmed *Escherichia coli* and *Klebsiella pneumoniae* BSI had concomitant gut colonization with matching strains of these bacteria (27). In line with this report, a prospective study of 9 pediatric allo-HCT patients matched BSI isolates to fecal samples (28). Another analysis of a large cohort of 708 allo-HCT patients with 4,768 fecal samples, found that gram-negative intestinal domination was associated with subsequent BSI both peaking 4–5 days post-transplantation. Together, these are consistent with the notion of a causal pathway of gram-negative BSI through mucosal injury and translocation (29).

Of note, exposure to fluoroquinolone prophylaxis has been associated with a lower risk of gram-negative BSI, but higher relative proportion of intestinal domination and BSI with *Escherichia coli* and specifically increased fluoroquinolone resistance (29). This suggests a role for antibiotic prophylaxis regimens in shaping microbiota composition (30) including intestinal domination and subsequent infection risk. In contrast, however, no reduction in microbiota diversity was observed in a comparison of microbiota composition of fecal samples collected during no-antibiotic vs levofloxacin-exposed intervals (31). In recently updated recommendations for antimicrobial prophylaxis (32), fluoroquinolone prophylaxis was recommended at least for recipients of myeloablative conditioning. The benefits and risks of such prescribing practices are matter of ongoing debate (33, 34) as antibiotic exposure has been associated with several adverse outcomes (35) including GVHD and GVHD-related mortality (36–41), infections (17, 29, 42, 43), antibiotic resistance (44, 45). Slavin *et al.* have pointed out (33) that the evidence base supporting antibacterial prophylaxis was accumulated prior to the widespread adoption of now-standard vigilant recognition of sepsis and immediate administration of empiric antibiotics, perhaps decreasing the rationale for prophylaxis in favor of other goals such as preservation of microbiome health and antimicrobial stewardship.

The relationship between the fungi that reside in the gut – that is, the *mycobiota* -- and risk of invasive fungal infection is less-well studied. In a proof of concept study, Zhai *et al.* showed that candidemia was preceded by rapid expansion of pathogenic *Candida spp.* in the gut, in striking similarity to the observations that have been made in bacteria (46).

Furthermore, intestinal *Candida spp.* domination was correlated with loss of bacterial diversity and exposure to activity against anaerobic bacteria. *Blautia producta* and *Bacteroides thetaiotaomicron* were used as markers for a healthy gut bacteria community and correlated inversely with fungal relative abundance, alluding to close interactions between bacterial microbiota and mycobiota. These observations reinforce again that a healthy microbiome may protect against infectious complication after allo-HCT such as enteric BSI, *Clostridioides difficile* (47) and respiratory viral infections (42, 43).

However, developing microbiota-based assays to predict and prevent BSI in allo-HCT recipients has several challenges 1) frequent fecal sampling would be necessary to sensitively predict BSI in allo-HCT patients because of a high microbiota volatility in the immediate period after transplant (21), 2) cut-off thresholds for specific strains may vary and 3) additional samples from other body sites might be necessary. A novel approach to predict infections may be offered by an assay of microbial cell-free DNA that is approved for clinical use in some locations in the U.S. (48). Use of this assay in adult and pediatric HCT recipients (49–52) suggests that it can be implemented in clinical practice and may offer a strategies to reduce use of broad-spectrum antibiotics and to spare the intestinal microbiome. However, larger studies to evaluate this approach are needed.

Microbial metabolites

Microbial metabolites - which are intermediate or end products of microbial metabolism derived from diet, host molecules, or directly from bacteria - influence host intestinal homeostasis, energy metabolism and immunity (53). These molecules can be profiled via mass spectrometry-based and chromatography-based techniques and their importance to microbiota-host interactions in general has been described in clinical and preclinical studies. A recent high-throughput metabolomic analysis of plasma metabolites in patients at the onset of aGVHD in comparison with transplant recipients who did not get GVHD (N=38 cases and N=62 controls were compared) showed significant variation in microbial metabolic pathways including those related to amino acids (tryptophan and arginine), lipids (lysoplasmalogens, plasmalogens, and phospholipids) and complex lipid products (medium- and long-chain fatty acid, polyunsaturated fatty acid and bile acids) (54).

In GVHD, the short-chain fatty acid (SCFA) butyrate is the most studied of the microbial metabolites. In mice, butyrate-producing bacteria have been shown to reduce GVHD (55, 56) and in humans, a higher abundance of butyrate-producing bacteria have been separately correlated with lack of exposure to antibiotics with less GVHD-related mortality (14) and fewer respiratory viral infection after allo-HCT (42, 43).

A recent retrospective study with prospective weekly stool collection from 316 patients compared patients sampled at aGVHD onset (N=35) with non-GVHD patients (N=35) and showed that fecal butyrate concentration was reduced at aGVHD onset in any stage of gastrointestinal (GI) aGVHD, while fecal concentrations of the SCFAs propionate and acetate were only reduced in severe GI aGVHD suggesting fecal butyrate may be a potential aGVHD marker for diagnosis and fecal propionate and acetate concentration for severity at the time of GVHD onset (57). This is in agreement with a previous study in pediatric allo-HCT patients, in which the authors reported lower fecal butyrate concentrations on day 14

were associated with aGVHD when they compared children who do developed GVHD and children who do not develop GVHD (41). Butyrate concentration in plasma was also measured in a clinical trial of human chorionic gonadotropin for steroid-refractory GVHD. Responders had higher plasma butyrate concentrations at study day 28 and 56 compared with baseline, while no change in butyrate concentration over time was observed in non-responders (58). In a case-control study that measured plasma butyrate concentrations per-day 100 after allo-HCT, lower plasma butyrate and propionate concentration were associated with the subsequent development of cGVHD (59). Interestingly, in another study increased relative abundance and presence of at least bacteria predicted to produce butyrate within 21 days after the onset of aGVHD was associated with developing steroid-refractory GVHD and cGVHD (60). In experiments using human intestinal organoids, the authors observed that butyrate inhibits colonic stem cells from forming an intact epithelial layer (60). One way to synthesize these disparate observations may be a model in which butyrate is protective before the onset of aGVHD perhaps at the level of modulating T cell biology, but after the onset of aGVHD it attenuates intestinal recovery by inhibiting colonic stem cells proliferation and healing. Another way may be dose dependency as high amounts of short-chain fatty acids fail to ameliorate GVHD in the mouse system (56).

Not only does the gut microbiome incur an injury during transplantation, but the oral communities are also disrupted. One study identified oral microbiota injury patterns and, recently the decreased production of polyamines by the microbiome was associated with higher rates of oral mucositis in a cohort of HCT recipients (61).

Collectively, these findings suggest that microbiota-derived metabolites may play a significant role the pathogenesis of GVHD, though more mechanistical studies need to be conducted.

Microbiota and GVHD pathogenesis

Mechanisms by which gut microbiota may influence GVHD are actively being investigated. Koyama *et al.* recently demonstrated in mouse studies that antigen presentation by intestinal epithelial cells to donor T cells plays a critical role in GVHD pathogenesis. The ability of intestinal epithelial cells to execute this was dependent on the MyD88/TRIF signaling pathway, indicating that it is induced by microbial signals. A study from the same group evaluated the impact of the peri-transplant gut microbiome on GVHD, making use of the endogenously dysbiotic microbiota of mice deficient in IL17RA (62), which has been shown to increase GVHD (63). Cohousing of wild-type mice with these mice was insufficient to increase GVHD in mice, however, cohousing posttransplant increased GVHD and continuously cohousing had an even bigger effect on GVHD, providing yet another example of the ability of microbiome to modulate GVHD severity.

Clinical strategies targeting the microbiome

The scientific and clinical challenges of engineering and implementing microbiota-based strategies into clinical practice are an active area of investigation in the field and are reviewed in detail (64). Several clinical studies are ongoing (Table 1) in the areas of GVHD

treatment and prevention and making use of diverse strategies including gut decontamination, prebiotics, probiotics, and optimization of antibiotic regimens.

Conclusions and future directions

Investigators continue to extend our understanding of the relationship between the intestinal microbiota and clinical outcomes after allo-HCT, and microbiota-associated GVHD pathogenesis including microbiota-based strategies. Numerous experiments have demonstrated manipulation of the gut microbiota can ameliorate or exacerbate GVHD severity in mice, indicating a causal relationship between microbes and alloreactivity. However, still unexplained is precisely how the microbiome modulates alloreactivity in mice and whether this occurs in humans. The role of intestinal microbiota is not only in GVHD but also in the context of graft-vs-tumor (65) as well as the role of microbial communities at other body site in allo-HCT related outcome (61) offer important avenues for future study.

Because of the complexity of influencing factors on intestinal microbiota and clinical outcome in patients undergoing allo-HCT randomized, prospective multi-center trials are essential and clinical trials will help to determine the role of microbiota-based strategies in clinical practice. Advances in diagnostic techniques and in the rational selection or design of probiotics may allow earlier and safer interventions. Additionally, more mouse studies using evolving techniques are needed to gain more insights in the molecular mechanisms.

Acknowledgements:

The authors would like to thank Kate A. Markey for helpful discussions.

Financial support and sponsorship:

This research was supported by NHLBI NIH Award K08HL143189, MSKCC Cancer Center Core Grant P30 CA008748, the Sawiris Foundation; the Society of Memorial Sloan Kettering Cancer Center; MSKCC Cancer Systems Immunology Pilot Grant, Empire Clinical Research Investigator Program, and Seres Therapeutics, the Society for MSK, and the American Society for Transplantation and Cellular Therapy. This research was supported by the Parker Institute for Cancer Immunotherapy at Memorial Sloan Kettering Cancer Center; J.U.P is a member of the Parker Institute for Cancer Immunotherapy; S.L. is supported by a research fellowship from the German Research Foundation (DFG, LI 3565/1-1).

References:

1. Markey KA, MacDonald KP, Hill GR. The biology of graft-versus-host disease: experimental systems instructing clinical practice. *Blood*. 2014;124(3):354–62. [PubMed: 24914137]
2. Andermann TM, Peled JU, Ho C, Reddy P, Riches M, Storb R, et al. The Microbiome and Hematopoietic Cell Transplantation: Past, Present, and Future. *Biol Blood Marrow Transplant*. 2018;24(7):1322–40. [PubMed: 29471034]
3. Koyama M, Hill GR. The primacy of gastrointestinal tract antigen-presenting cells in lethal graft-versus-host disease. *Blood*. 2019;134(24):2139–48. [PubMed: 31697827]
4. Fredricks DN. The gut microbiota and graft-versus-host disease. *J Clin Invest*. 2019;129(5):1808–17. [PubMed: 31042160]
5. Ferrara JLM, Chaudhry MS. GVHD: biology matters. *Blood Adv*. 2018;2(22):3411–7. [PubMed: 30482771]
6. Shono Y, van den Brink MRM. Gut microbiota injury in allogeneic haematopoietic stem cell transplantation. *Nature Reviews Cancer*. 2018;18(5):283–95. [PubMed: 29449660]

7. Zeiser R, Socié G, Blazar BR. Pathogenesis of acute graft-versus-host disease: from intestinal microbiota alterations to donor T cell activation. *Br J Haematol.* 2016;175(2):191–207. [PubMed: 27619472]
8. Rafei H, Jenq RR. Microbiome-intestinal crosstalk during acute graft-versus-host disease (GVHD). *Blood.* 2020.
9. 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. [PubMed: 32101664] First multicenter study showing that microbiota disruption during allo-HCT is similar across transplantation centers and geographic locations and that microbiota diversity during day 7–21 is a predictor of mortality after allo-HCT.
10. 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. [PubMed: 31566729]
11. Han L, Zhang H, Chen S, Zhou L, Li Y, Zhao K, et al. Intestinal Microbiota Can Predict Acute Graft-versus-Host Disease Following Allogeneic Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant.* 2019;25(10):1944–55. [PubMed: 31299215]
12. Han L, Zhao K, Li Y, Han H, Zhou L, Ma P, et al. A gut microbiota score predicting acute graft-versus-host disease following myeloablative allogeneic hematopoietic stem cell transplantation. *Am J Transplant.* 2020;20(4):1014–27. [PubMed: 31605563] This paper describes a risk score that uses 20 features of intestinal bacteria to predict GVHD. The authors used a discovery and validation cohort.
13. Golob JL, Pergam SA, Srinivasan S, Fiedler TL, Liu C, Garcia K, et al. Stool Microbiota at Neutrophil Recovery Is Predictive for Severe Acute Graft vs Host Disease After Hematopoietic Cell Transplantation. *Clin Infect Dis.* 2017;65(12):1984–91. [PubMed: 29020185]
14. 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. [PubMed: 25977230]
15. 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. [PubMed: 26209659]
16. Holler E, Butzhammer P, Schmid K, Hundsruker 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. [PubMed: 24492144]
17. Taur Y, Xavier JB, Lipuma L, Ubada C, Goldberg J, Gobourne A, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis.* 2012;55(7):905–14. [PubMed: 22718773]
18. Ubada C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, et al. Vancomycin-resistant *Enterococcus* domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest.* 2010;120(12):4332–41. [PubMed: 21099116]
19. 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. [PubMed: 31780560] Human and mouse study showing that *Enterococcus* domination occurs frequently after allo-HCT, exacerbates GVHD, and is driven by dietary lactose.
20. 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.
21. Morjaria S, Schluter J, Taylor BP, Littmann ER, Carter RA, Fontana E, et al. Antibiotic-Induced Shifts in Fecal Microbiota Density and Composition during Hematopoietic Stem Cell Transplantation. *Infect Immun.* 2019;87(9).
22. Dubin KA, Mathur D, McKenney PT, Taylor BP, Littmann ER, Peled JU, et al. Diversification and Evolution of Vancomycin-Resistant *Enterococcus faecium* during Intestinal Domination. *Infect*

- Immun. 2019;87(7).A human and mouse study showing that apparently stable VRE domination as detected by 16S rRNA is highly complex and evolves genetically over time.
23. Zlitni S, Bishara A, Moss EL, Tkachenko E, Kang JB, Culver RN, et al. Strain-resolved microbiome sequencing reveals mobile elements that drive bacterial competition on a clinical timescale. *Genome Med.* 2020;12(1):50. [PubMed: 32471482] Human study revealing strain heterogeneity with increased antibiotic resistance in apparently stable taxonomic microbiota composition over the course of allo-HCT.
 24. Rashidi A, Ebadi M, Shields-Cutler RR, Kruziki K, Manias DA, Barnes AMT, et al. Early *E. casseliflavus* gut colonization and outcomes of allogeneic hematopoietic cell transplantation. *PLoS One.* 2019;14(8):e0220850. [PubMed: 31393924]
 25. Rashidi A, Ebadi M, Shields-Cutler RR, DeFor TE, Al-Ghalith GA, Ferrieri P, et al. Pretransplant Gut Colonization with Intrinsically Vancomycin-Resistant Enterococci (*E. gallinarum* and *E. casseliflavus*) and Outcomes of Allogeneic Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant.* 2018;24(6):1260–3. [PubMed: 29407252]
 26. Kim SG, Becattini S, Moody TU, Shliaha PV, Littmann ER, Seok R, et al. Microbiota-derived lantibiotic restores resistance against vancomycin-resistant Enterococcus. *Nature.* 2019;572(7771):665–9. [PubMed: 31435014] Human and mouse study demonstrating that lantibiotic-producing commensal strains reduce colonization by VRE.
 27. Tamburini FB, Andermann TM, Tkachenko E, Senchyna F, Banaei N, Bhatt AS. Precision identification of diverse bloodstream pathogens in the gut microbiome. *Nat Med.* 2018;24(12):1809–14. [PubMed: 30323331]
 28. Kelly MS, Ward DV, Severyn CJ, Arshad M, Heston SM, Jenkins K, et al. Gut Colonization Preceding Mucosal Barrier Injury Bloodstream Infection in Pediatric Hematopoietic Stem Cell Transplantation Recipients. *Biol Blood Marrow Transplant.* 2019;25(11):2274–80. [PubMed: 31326608] Single center study reporting that gut colonization and expansion of bacterial strains causing mucosal barrier injury BSI in allo-HCT recipients occur before BSI onset.
 29. Stoma I, Littmann ER, Peled JU, Giral S, van den Brink MRM, Pamer EG, et al. Compositional flux within the intestinal microbiota and risk for bloodstream infection with gram-negative bacteria. *Clin Infect Dis.* 2020. Single center study reporting that gut colonization with gram-negative bacteria is highly predictive of BSI in patients undergoing allo-HCT and influenced by fluoroquinolone prophylaxis.
 30. Bekker V, Zwitter RD, Knetsch CW, Sanders I, Berghuis D, Heidt PJ, et al. Dynamics of the Gut Microbiota in Children Receiving Selective or Total Gut Decontamination Treatment during Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant.* 2019;25(6):1164–71. [PubMed: 30731251]
 31. Rashidi A, Kaiser T, Holtan SG, Rehman TU, Weisdorf DJ, Khoruts A, et al. Levaquin Gets a Pass. *Biol Blood Marrow Transplant.* 2020;26(4):778–81. [PubMed: 31870930]
 32. Taplitz RA, Kennedy EB, Bow EJ, Crews J, Gleason C, Hawley DK, et al. Antimicrobial Prophylaxis for Adult Patients With Cancer-Related Immunosuppression: ASCO and IDSA Clinical Practice Guideline Update. *Journal of Clinical Oncology.* 2018;36(30):3043–54. [PubMed: 30179565]
 33. Slavin MA, Worth LJ, Seymour JF, Thursky KA. Better Sepsis Management Rather Than Fluoroquinolone Prophylaxis for Patients With Cancer-Related Immunosuppression. *Journal of Clinical Oncology.* 2019;37(13):1139–40. [PubMed: 30844318]
 34. Taplitz RA, Kennedy EB, Flowers CR. Reply to M.A. Slavin et al. *Journal of Clinical Oncology.* 2019;37(13):1140–1. [PubMed: 30844317]
 35. Gavrilaki M, Sakellari I, Anagnostopoulos A, Gavrilaki E. The impact of antibiotic-mediated modification of the intestinal microbiome on outcomes of allogeneic hematopoietic cell transplantation: systematic review and meta-analysis. *Biol Blood Marrow Transplant.* 2020.
 36. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. *Sci Transl Med.* 2016;8(339):339ra71.
 37. Weber D, Jenq RR, Peled JU, Taur Y, Hiergeist A, Koestler J, et al. Microbiota Disruption Induced by Early Use of Broad-Spectrum Antibiotics Is an Independent Risk Factor of Outcome after

- Allogeneic Stem Cell Transplantation. *Biol Blood Marrow Transplant*. 2017;23(5):845–52. [PubMed: 28232086]
38. Nishi K, Kanda J, Hishizawa M, Kitano T, Kondo T, Yamashita K, et al. Impact of the Use and Type of Antibiotics on Acute Graft-versus-Host Disease. *Biol Blood Marrow Transplant*. 2018;24(11):2178–83. [PubMed: 30417828]
 39. Lee SE, Lim JY, Ryu DB, Kim TW, Park SS, Jeon YW, et al. Alteration of the Intestinal Microbiota by Broad-Spectrum Antibiotic Use Correlates with the Occurrence of Intestinal Graft-versus-Host Disease. *Biol Blood Marrow Transplant*. 2019;25(10):1933–43. [PubMed: 31195137]
 40. Simms-Waldrup 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 Graft-versus-Host Disease in Pediatric Stem Cell Transplantation Patients. *Biol Blood Marrow Transplant*. 2017;23(5):820–9. [PubMed: 28192251]
 41. Romick-Rosendale LE, Haslam DB, Lane A, Denson L, Lake K, Wilkey A, et al. Antibiotic Exposure and Reduced Short Chain Fatty Acid Production after Hematopoietic Stem Cell Transplant. *Biol Blood Marrow Transplant*. 2018;24(12):2418–24. [PubMed: 30055351]
 42. Ogimi C, Krantz EM, Golob JL, Waghmare A, Liu C, Leisenring WM, et al. Antibiotic Exposure Prior to Respiratory Viral Infection Is Associated with Progression to Lower Respiratory Tract Disease in Allogeneic Hematopoietic Cell Transplant Recipients. *Biol Blood Marrow Transplant*. 2018;24(11):2293–301. [PubMed: 29777867]
 43. Haak BW, Littmann ER, Chaubard JL, Pickard AJ, Fontana E, Adhi F, et al. Impact of gut colonization with butyrate-producing microbiota on respiratory viral infection following allo-HCT. *Blood*. 2018;131(26):2978–86. [PubMed: 29674425]
 44. Macesic N, Morrissey CO, Cheng AC, Spencer A, Peleg AY. Changing microbial epidemiology in hematopoietic stem cell transplant recipients: increasing resistance over a 9-year period. *Transpl Infect Dis*. 2014;16(6):887–96. [PubMed: 25298044]
 45. Hauck CG, Chong PP, Miller MB, Jamieson K, Fine JP, Foster MC, et al. Increasing Rates of Fluoroquinolone Resistance in *Escherichia coli* Isolated From the Blood and Urine of Patients with Hematologic Malignancies and Stem Cell Transplant Recipients. *Pathog Immun*. 2016;1(2):234–42. [PubMed: 28004038]
 46. Zhai B, Ola M, Rolling T, Tosini NL, Jshowitz S, Littmann ER, et al. High-resolution mycobiota analysis reveals dynamic intestinal translocation preceding invasive candidiasis. *Nat Med*. 2020;26(1):59–64. [PubMed: 31907459] First study showing that intestinal expansion of pathogenic *Candida* species preceds candidemia in allo-HCT patients and is associated with loss of bacterial diversity.
 47. Lee YJ, Arguello ES, Jenq RR, Littmann E, Kim GJ, Miller LC, et al. Protective Factors in the Intestinal Microbiome Against *Clostridium difficile* Infection in Recipients of Allogeneic Hematopoietic Stem Cell Transplantation. *J Infect Dis*. 2017;215(7):1117–23. [PubMed: 28498996]
 48. Goggin KP, Gonzalez-Pena V, Inaba Y, Allison KJ, Hong DK, Ahmed AA, et al. Evaluation of Plasma Microbial Cell-Free DNA Sequencing to Predict Bloodstream Infection in Pediatric Patients With Relapsed or Refractory Cancer. *JAMA Oncol*. 2019.
 49. Fung M, Zompi S, Seng H, Hollemon D, Parham A, Hong DK, et al. Plasma Cell-Free DNA Next-Generation Sequencing to Diagnose and Monitor Infections in Allogeneic Hematopoietic Stem Cell Transplant Patients. *Open Forum Infect Dis*. 2018;5(12):ofy301. [PubMed: 30581881]
 50. Camargo JF, Ahmed AA, Lindner MS, Morris MI, Anjan S, Anderson AD, et al. Next-generation sequencing of microbial cell-free DNA for rapid noninvasive diagnosis of infectious diseases in immunocompromised hosts. *F1000Res*. 2019;8:1194. [PubMed: 31814964]
 51. Armstrong AE, Rossoff J, Hollemon D, Hong DK, Muller WJ, Chaudhury S. Cell-free DNA next-generation sequencing successfully detects infectious pathogens in pediatric oncology and hematopoietic stem cell transplant patients at risk for invasive fungal disease. *Pediatr Blood Cancer*. 2019;66(7):e27734. [PubMed: 30941906]
 52. Steinbrink JM, Hong DK, Bergin SP, Al-Rohil RN, Perfect JR, Maziarz EK. The robust and rapid role of molecular testing in precision fungal diagnostics: A case report. *Med Mycol Case Rep*. 2020;27:77–80. [PubMed: 32149044]

53. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nature Reviews Immunology*. 2016;16(6):341–52.
54. Michonneau D, Latis E, Curis E, Dubouchet L, Ramamoorthy S, Ingram B, et al. Metabolomics analysis of human acute graft-versus-host disease reveals changes in host and microbiota-derived metabolites. *Nat Commun*. 2019;10(1):5695. [PubMed: 31836702] First study profiling plasma metabolites from HLA-identical related recipient and donor pairs supporting that microbiota-derived metabolites influence allogeneic T cell reactivity.
55. Mathewson ND, Jenq R, Mathew AV, Koenigsnecht 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. [PubMed: 26998764]
56. Fujiwara H, Docampo MD, Riwe M, Peltier D, Toubai T, Henig I, et al. Microbial metabolite sensor GPR43 controls severity of experimental GVHD. *Nat Commun*. 2018;9(1):3674. [PubMed: 30201970]
57. Payen M, Nicolis I, Robin M, Michonneau D, Delannoye J, Mayeur C, et al. Functional and phylogenetic alterations in gut microbiome are linked to graft-versus-host disease severity. *Blood Adv*. 2020;4(9):1824–32. [PubMed: 32353108] Single center study using fecal SFCA profiles, 16S rRNA gene sequencing and real-time quantitative PCR identified that microbiota changes were highly specific of aGVHD severity characterized by loss of anaerobic bacteria and decrease in fecal SCFA concentration.
58. Holtan SG, Hoeschen AL, Cao Q, Arora M, Bachanova V, Brunstein CG, et al. Facilitating resolution of life-threatening acute GVHD with human chorionic gonadotropin and epidermal growth factor. *Blood Adv*. 2020;4(7):1284–95. [PubMed: 32236525] In a phase 1 study investigating urinary-derived human chorionic gonadotropin treatment in allo-HCT recipients with GVHD and reporting plasma butyrate concentration as a marker for mucosal recovery.
59. Markey KA, Schluter J, Gomes AL, Littmann E, Pickard A, Taylor BP, et al. Microbe-derived short chain fatty acids butyrate and propionate are associated with protection from chronic GVHD. *Blood*. 2020. First study investigating the relationship of the intestinal microbiota with cGVHD after allo-HCT and the authors report lower concentrations of propionate and butyrate in day 100 plasma samples from patients who developed cGVHD compared with those who did not.
60. Golob JL, DeMeules MM, Loeffelholz T, Quinn ZZ, Dame MK, Silvestri SS, et al. Butyrogenic bacteria after acute graft-versus-host disease (GVHD) are associated with the development of steroid-refractory GVHD. *Blood Adv*. 2019;3(19):2866–9. [PubMed: 31585950] Single center study showing that abundance of butyrate-producing bacteria after the onset of aGVHD is associated with subsequent steroid-refractory or cGVHD.
61. Shouval R, Eshel A, Dubovski B, Kuperman AA, Danylesko I, Fein JA, et al. Patterns of salivary microbiota injury and oral mucositis in recipients of allogeneic hematopoietic stem cell transplantation. *Blood Advances*. 2020;4(13):2912–7. [PubMed: 32598476]
62. Bowerman KL, Varelias A, Lachner N, Kuns RD, Hill GR, Hugenholtz P. Continuous pre- and post-transplant exposure to a disease-associated gut microbiome promotes hyper-acute graft-versus-host disease in wild-type mice. *Gut Microbes*. 2020:1–17.
63. Varelias A, Ormerod KL, Bunting MD, Koyama M, Gartlan KH, Kuns RD, et al. Acute graft-versus-host disease is regulated by an IL-17-sensitive microbiome. *Blood*. 2017;129(15):2172–85. [PubMed: 28137828]
64. Markey KA, van den Brink MRM, Peled JU. Therapeutics Targeting the Gut Microbiome: Rigorous Pipelines for Drug Development. *Cell Host Microbe*. 2020;27(2):169–72. [PubMed: 32053786]
65. 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. [PubMed: 28296584]
66. Koyama M, Mukhopadhyay P, Schuster IS, Henden AS, Hulsdunker J, Varelias A, et al. MHC Class II Antigen Presentation by the Intestinal Epithelium Initiates Graft-versus-Host Disease and Is Influenced by the Microbiota. *Immunity*. 2019;51(5):885–98.e7. [PubMed: 31542340] Mouse study showing that intestinal microbiota influenced MHC class II expression by IEC and initiated of lethal GVHD in mice.

Key points:

- Intestinal microbiota disruption occurs in patients undergoing allo-HCT across transplant centers and geographic locations; higher diversity during the periengraftment period is predictive of better outcome
- Enterococcus domination is common after allo-HCT, is promoted by dietary lactose and accelerates GHVD in mice
- Bacterial and fungal bloodstream infections in allo-HCT recipients are preceded by intestinal colonization with corresponding organisms, supporting the gut as a source for disseminated infection
- Microbiota-derived metabolites may mitigate or promote GVHD
- MHC class II expression and antigen presentation by intestinal epithelial cells is influenced by peritransplant intestinal microbiota, this is responsible for the initiation of lethal GVHD in mice

Table 1

Ongoing clinical trials

Study title	Intervention	Primary endpoint	NCT number
GVHD treatment			
Fecal Microbiota Transplantation for Steroid Resistant/Dependent Acute GI GVHD (FEMITGIGVHD)	• FMT	• Response rate of GI aGVHD	NCT03812705
Fecal Microbiota Transplantation (FMT) in Recipients After Allogeneic Hematopoietic Cell Transplantation (HCT)	• FMT	• Gut microbiome diversity	NCT03720392
Fecal Microbiota Transplantation in aGvHD After ASCT	• FMT	• GI aGvHD remission	NCT03819803
Treatment of Steroid Refractory Gastro-intestinal Acute GVHD after Allogeneic HSCT With Fecal Microbiota transfer (HERACLES)	• FMT	• GI and overall aGVHD response	NCT03359980
FMT In High-Risk Acute GVHD After ALLO HCT	• FMT	• Proportion of patients who are able to swallow the specified number of capsules	NCT04139577
Fecal Microbiota Transplantation With Ruxolitinib and Steroids as an Upfront Treatment of Severe Acute Intestinal GVHD (JAK-FMT)	• FMT • Ruxolitinib	• Overall survival	NCT04269850
Fecal Microbiota Transplantation for Steroid Resistant and Steroid Dependent Gut Acute Graft Versus Host Disease	• FMT	• Serious adverse events	NCT03214289
Fecal Microbiota Transplantation For The Treatment Of Gastro-Intestinal Acute GVHD	• FMT	• Toxicity • Efficacy	NCT04059757
GVHD prevention			
<i>Gut decontamination</i>			
Gut decontamination in pediatric allogeneic hematopoietic	• Vancomycin-polymyxin B	• Fecal microbiota diversity	NCT02641236
<i>Probiotic</i>			
Lactobacillus plantarum in preventing acute graft versus host disease in children undergoing donor stem cell transplant	• Lactobacillus plantarum strain 299 and 299v	• Incidence of stage 1–4 GI aGVHD	NCT03057054
CBM588 in Improving Clinical Outcomes in Participants Who Have Undergone Donor Hematopoietic Stem Cell Transplant	• Clostridium butyricum CBM 588 Probiotic Strain	• Incidence of adverse events • Feasibility	NCT03922035
<i>Prebiotic</i>			
Dietary Manipulation of the Microbiome-metabolomic Axis for Mitigating GVHD in Allo HCT Patients	• Potato-based starch	• Incidence of grade II-IV aGVHD	NCT02763033
Human Lysozyme Goat Milk for the Prevention of Graft Versus Host Disease in Patients With Blood Cancer Undergoing a Donor Stem Cell Transplant	• Human Lysozyme Goat Milk	• Patients' ability to drink the specified daily amount human lysozyme goat milk	NCT04177004

Study title	Intervention	Primary endpoint	NCT number
		<ul style="list-style-type: none"> • Unacceptable toxicity • Adverse events • Volume of human lysozyme goat milk consumed 	
The Use of A Prebiotic to Promote a Healthy Gut Microbiome in Pediatric Stem Cell Transplant Recipients	<ul style="list-style-type: none"> • Inulin 	<ul style="list-style-type: none"> • Change in α- and β-bacterial diversity measures in stool • Change in fecal short chain fatty acid concentration 	NCT04111471
Oral Supplementation of 2'-Fucosyllactose in Allogeneic Bone Marrow Transplant Recipients	<ul style="list-style-type: none"> • 2'-fucosyllactose 	<ul style="list-style-type: none"> • BSI • Number of patients able to take 2'-fucosyllactose 	NCT04263597
<i>Infection prophylaxis</i>			
Rifaximin for Infection Prophylaxis in Hematopoietic Stem Cell Transplantation	<ul style="list-style-type: none"> • Rifaximin 	<ul style="list-style-type: none"> • Fecal microbiota diversity 	NCT03529825
Choosing the Best Antibiotic to Protect Friendly Gut Bacteria During the Course of Stem Cell Transplant	<ul style="list-style-type: none"> • Piperacillin-tazobactam vs cefepime 	<ul style="list-style-type: none"> • Change in Clostridiales abundance 	NCT03078010
Optimization of Antibiotic Treatment in Hematopoietic Stem Cell Receptors (Optimbioma)	<ul style="list-style-type: none"> • Optimization/antibiotic strategy 	<ul style="list-style-type: none"> • Impact on microbiota 	NCT03727113
Autologous Fecal Microbiota Transplantation (Auto-FMT) for Prophylaxis of Clostridium Difficile Infection in Recipients of Allogeneic Hematopoietic Stem Cell Transplantation	<ul style="list-style-type: none"> • FMT 	<ul style="list-style-type: none"> • Clostridioides difficile infection 	NCT02269150