Anaerobic Antibiotics and the Risk of Graft-Versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation

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Abstract

Certain anaerobic bacteria are important for maintenance of gut barrier integrity and immune tolerance and may influence the risk of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). We conducted a single-center retrospective...
cohort study of allogeneic HSCT recipients to evaluate associations between receipt of antibiotics with an anaerobic spectrum of activity and GVHD outcomes. We identified 1214 children and adults who developed febrile neutropenia between 7 days before and 28 days after HSCT and compared GVHD risk and mortality among patients who received anaerobic antibiotics (piperacillin-tazobactam or carbapenems; n=491) to patients who received only antibiotics with minimal activity against anaerobes (aztreonam, cefepime, or ceftazidime; n=723). We performed metagenomic sequencing of serial fecal samples from 36 pediatric patients to compare the effects of specific antibiotics on the gut metagenome. Receipt of anaerobic antibiotics was associated with higher hazards of acute gut/liver GVHD (hazard ratio: 1.26; 95% confidence interval: 1.03–1.54) and acute GVHD mortality (hazard ratio: 1.63; 95% confidence interval: 1.08–2.46), but not chronic GVHD diagnosis (hazard ratio: 1.04; 95% confidence interval: 0.84–1.28) or chronic GVHD mortality (hazard ratio: 0.88; 95% confidence interval: 0.53–1.45). Anaerobic antibiotics resulted in decreased gut bacterial diversity, reduced abundances of Bifidobacteriales and Clostridiales, and loss of bacterial genes encoding butyrate biosynthesis enzymes from the gut metagenome. Acute gut/liver GVHD was preceded by a sharp decline in bacterial butyrate biosynthesis genes with antibiotic treatment. Our findings demonstrate that exposure to anaerobic antibiotics is associated with increased risks of acute gut/liver GVHD and acute GVHD mortality after allogeneic HSCT. Use of piperacillin-tazobactam or carbapenems should be reserved for febrile neutropenia cases in which anaerobic or multidrug-resistant infections are suspected.

**Keywords**

febrile neutropenia; piperacillin-tazobactam; carbapenems; gut microbiome

**INTRODUCTION**

Febrile neutropenia occurs in 85–100% of patients who undergo allogeneic hematopoietic stem cell transplantation (HSCT).1,2 Current guidelines from the Infectious Diseases Society of America and the National Comprehensive Cancer Network recommend that hospitalized patients with anticipated prolonged and severe neutropenia who develop fever should receive an empirical broad-spectrum antibiotic with antipseudomonal activity, such as piperacillin-tazobactam, cefepime, ceftazidime, or a carbapenem (imipenem-cilastatin or meropenem).3 Although each of these antibiotics may affect the gut microbiota, only piperacillin-tazobactam and carbapenems have appreciable activity against anaerobic bacteria.4,5 Recent studies suggest that receipt of these antibiotics during episodes of febrile neutropenia may increase the risk of graft-versus-host disease (GVHD) after allogeneic HSCT.6–8 Additionally, lower abundances of certain commensal gut anaerobes, and particularly bacteria from the order Clostridiales, have been associated with the development of GVHD.8–10 Many Clostridiales species produce butyrate—a short-chain fatty acid that is a preferred nutrient source for colonocytes and that is increasingly recognized to be important for maintenance of gut barrier integrity and immune tolerance.11–13 Taken together, these data suggest that anaerobic antibiotics may increase the risk of GVHD after allogeneic HSCT through depletion of intestinal butyrate.
In this study, we identified 1214 children and adults who developed febrile neutropenia after allogeneic HSCT and evaluated associations between receipt of broad-spectrum antibiotics and GVHD outcomes. Using shotgun metagenomic sequencing of serial fecal samples from 36 children and adolescents in this cohort, we determined the effect of these antibiotics on the diversity and composition of the gut microbiota and the relative abundance of bacterial genes for butyrate biosynthesis in the gut metagenome.

MATERIALS AND METHODS

Study population

We performed a retrospective cohort study of patients undergoing their first allogeneic HSCT through the Duke Pediatric Blood and Marrow Transplant or Duke Hematologic Malignancies and Cellular Therapy Programs between January 1, 2005 and December 31, 2016. We excluded patients who received T-cell-depleted grafts or who died within the first 28 days after HSCT. The study protocol was approved by the Duke University Institutional Review Board.

Transplantation practices

Prior to February 2015, ciprofloxacin and metronidazole prophylaxis was routinely given to adult patients from the start of conditioning through the duration of neutropenia or initiation of intravenous broad-spectrum antibiotics. In February 2015, prophylaxis with ciprofloxacin alone became standard practice for adult patients. Routine antibacterial prophylaxis was not used for pediatric patients throughout the study period. For Pneumocystis jirovecii prophylaxis, pediatric and adult patients received trimethoprim-sulfamethoxazole from the start of conditioning through two days before HSCT, followed by trimethoprim-sulfamethoxazole, inhaled pentamidine, or intravenous pentamidine starting 30 days after HSCT. Initial therapy for grade II-IV acute GVHD or moderate to severe chronic GVHD was systemic corticosteroids throughout the study period. Oral budesonide was often added for patients with gastrointestinal involvement.

Data sources and measures

We extracted patient demographics and HSCT characteristics from secure databases maintained by the transplant programs. For acute GVHD, stage and grade were assigned using the criteria proposed by Przepiorka et al. For chronic GVHD, stage and grade were assigned using the criteria established by the 2014 National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD. Treating transplant providers recorded diagnoses of acute and chronic GVHD based on clinical symptoms and, when obtained, biopsy results. We considered GVHD to be the cause of death if it was classified by the transplant physician as the primary cause of death or as a secondary cause of death in a patient who received treatment for GVHD within 30 days of death from an infection. We identified all antibiotics received by patients between 7 days before and 28 days after allogeneic HSCT using medication data extracted from electronic medical records.
Statistical analyses

The analyses presented herein were limited to patients who received treatment for febrile neutropenia between 7 days before and 28 days after HSCT and were administered an antibiotic with an anaerobic spectrum of activity (piperacillin-tazobactam or a carbapenem) or only antibiotics without appreciable anaerobic activity (aztreonam, cefepime, or ceftazidime). Patients who were diagnosed with GVHD prior to onset of febrile neutropenia were excluded. We used Chi-square tests or two-sample t-tests to evaluate for differences in patient and HSCT characteristics in these groups. We used a Cox proportional hazards model to evaluate for an association between anaerobic antibiotics and our primary outcome, acute gut/liver GVHD. We similarly used Cox proportional hazards models to evaluate associations between receipt of anaerobic antibiotics and the following secondary outcomes: acute skin GVHD, acute GVHD mortality, chronic GVHD, and chronic GVHD mortality. We adjusted all models for the following potential confounding factors: year of HSCT, age, sex, race, transplant indication, HSCT donor, HSCT source, human leukocyte antigen (HLA) matching, conditioning regimen intensity, and GVHD prophylaxis regimen. Time at risk was from the date of HSCT until death from any cause or censoring. Patients were censored 1 year after HSCT for acute GVHD outcomes and 5 years after HSCT for chronic GVHD outcomes. The proportional hazards assumption was verified for each model using Schoenfeld residuals and an extended Cox model.

For the models for which mortality was an outcome, we also performed a competing risks analysis with a subdistribution hazard function. However, the findings did not substantively differ from those obtained with Cox proportional hazards models. These analyses also showed that antibiotic group was not associated with mortality from causes other than GVHD.

Metagenomic data analyses

A subset of children and adolescents (<18 years) included in these analyses were enrolled in a prospective cohort study that began in October 2015. Subjects were recruited during the pre-transplant evaluation and were followed until 100 days after HSCT. For the present analyses, we identified 36 subjects who developed febrile neutropenia between 7 days before and 28 days after HSCT and received either an anaerobic antibiotic (piperacillin-tazobactam or meropenem) or cefepime. For each subject, we selected one fecal sample collected within the 10 days prior to the initiation of antibiotics and a second fecal sample obtained while on antibiotic therapy for at least 48 hours. We extracted DNA using MagAttract PowerSoil DNA EP kits (Qiagen, Germantown, MD). We then constructed DNA sequencing libraries using Nextera XT DNA Library Prep Kits (Illumina Inc., San Diego, CA) and sequenced these libraries on a NextSeq500 Sequencing System as 150-base paired-end reads. We trimmed reads and removed host decontamination using Trimmomatic and Bowtie2. Host-decontaminated reads were profiled for bacterial species abundances using MetaPhlAn2. We aligned sequencing reads with a publicly available reference database to identify bacterial genes encoding butyrate biosynthesis enzymes. We used Wilcoxon signed-rank tests to compare the Shannon diversity and Chao1 richness of paired fecal samples obtained before and during treatment with antibiotics. We compared the bacterial composition of paired fecal samples using PERMANOVA and Bray-Curtis distances. We
used Wilcoxon signed-rank tests to compare the relative abundances of bacterial orders and genera in paired samples collected prior to and during antibiotic treatment. Trimmed and host-decontaminated metagenomic reads were then profiled for the abundance of genes involved in butyrate biosynthesis using ShortBRED.\textsuperscript{20} Finally, we used Wilcoxon signed-rank tests to compare the abundances of genes involved in butyrate biosynthesis and detected by metagenomic sequencing in paired fecal samples across patient groups.

**Data Sharing Statement**

The metagenomic sequencing dataset supporting the conclusions of this study is available in the Sequence Read Archive (PRJNA588783). The statistical files and script used for data analyses are also publicly available (https://github.com/mskelly7/Tanaka_GVHD_manuscript).

**RESULTS**

**Patient characteristics**

Of 1214 patients with febrile neutropenia, 723 (60\%) received aztreonam, cefepime, or ceftazidime only and 491 (40\%) received piperacillin-tazobactam or a carbapenem (Table 1). Compared with patients who received aztreonam, cefepime, or ceftazidime, patients who received an anaerobic antibiotic were older ($P<0.0001$), more likely to have a hematological malignancy ($P<0.0001$), and more often had a peripheral blood donor source ($P<0.0001$). Median [interquartile range (IQR)] duration of antibiotics for febrile neutropenia in the aztreonam/cefepime/ceftazidime group was 17 (8, 25) days. Median (IQR) duration of antibiotics for febrile neutropenia in the anaerobic antibiotic group was 15 (8, 23) days, including 12 (7, 18) days on which an anaerobic antibiotic was received. Of 491 patients in the anaerobic antibiotic group, 293 (60\%) received piperacillin-tazobactam and 275 (56\%) received a carbapenem (Table 2).

**GVHD outcomes**

Of the 1214 patients, 511 (42\%) developed acute GVHD only, 88 (7\%) developed chronic GVHD only, and 335 (28\%) developed both acute and chronic GVHD. Overall, 270 of 491 (55\%) patients who received anaerobic antibiotics were diagnosed with grade II-IV acute GVHD compared to 337 of 723 (46\%) patients who received aztreonam, cefepime, or ceftazidime. Details regarding organ involvement and stages of acute GVHD diagnoses by antibiotic group are shown in Supplemental Table 1. Four hundred thirty-one (36\%) patients were diagnosed with acute GVHD of the gut or liver (Table 3). Cumulative incidence curves for GVHD outcomes by antibiotic group are shown in Figure 1. In multivariable analyses, receipt of an anaerobic antibiotic regimen was associated with a higher hazard of acute GVHD of the gut or liver [hazard ratio (HR): 1.26; 95\% confidence interval (CI): 1.03–1.54] and 1-year acute GVHD mortality (HR: 1.63; 95\% CI: 1.08–2.46). Receipt of anaerobic antibiotics was not associated with acute skin GVHD (HR: 1.06; 95\% CI: 0.86–1.31), chronic GVHD diagnosis (HR: 1.04; 95\% CI: 0.84–1.28), or 5-year chronic GVHD mortality (HR: 0.88; 95\% CI: 0.53–1.43). The hazard ratios for these analyses were similar when patients from the pediatric and adult HSCT programs were analyzed separately (Supplemental Table 2).
Effect of antibiotics on the diversity and composition of the gut microbiota

Of the 36 children and adolescents included in this analysis, 26 received cefepime and 10 received piperacillin-tazobactam or meropenem (Supplemental Table 3). There were no culture-proven infections that corresponded with the timing of initiation of antibiotics in these 36 subjects. Fecal samples obtained following initiation of antibiotic therapy were collected after a median (IQR) of 10 (6, 27.5) days among subjects receiving cefepime and a median (IQR) of 14.5 (10, 22) days among subjects receiving piperacillin-tazobactam or meropenem (Wilcoxon rank-sum test; P=0.30). No subject received clindamycin, metronidazole, or another antibiotic with substantial anaerobic activity prior to fecal sample collection. A total of 637,698,091 high-quality metagenomic sequences (mean of 8,856,918 sequences per sample) were obtained from the 72 fecal samples included in this analysis. Sequences were assigned to 284 bacterial species representing 116 genera from 27 orders and six phyla. Among subjects receiving anaerobic antibiotics, median (IQR) Shannon diversity declined from 2.0 (1.6, 2.5) prior to antibiotics to 0.9 (0.3, 1.2) while on antibiotic therapy (P=0.01) (Supplemental Figure 1). Median (IQR) Chao1 richness also declined from 28 (19, 49) to 5 (3, 15) with exposure to anaerobic antibiotics (P=0.01). By comparison, cefepime treatment was not associated with changes in gut bacterial diversity or species richness. Bacterial composition differed in samples collected before and during anaerobic antibiotic therapy (P<0.001) and before and during treatment with cefepime (P=0.01). Cefepime and anaerobic antibiotics were associated with distinct changes in the relative abundances of bacterial orders (Figure 2 and Supplemental Figure 2). Cefepime treatment was associated with a decline in the relative abundances of Enterobacterales (P=0.01) and Lactobacillales (P=0.04). In contrast, anaerobic antibiotic therapy was associated with a decline in the relative abundances of Bifidobacteriales (P=0.01) and Clostridiales (P=0.002) and an increase in the relative abundance of Lactobacillales (P=0.01). Several genera of Clostridiales declined in abundance during treatment with anaerobic antibiotics (Table 4), including genera that contain known butyrate-producing species (Blautia, Clostridium, and Lachnoclostridium). In addition, Bifidobacterium was among the most abundant genera in fecal samples prior to antibiotic therapy but was not identified in any samples collected during anaerobic antibiotic treatment.

Genes for butyrate biosynthesis and acute GVHD of the gut or liver

The relative abundance of Clostridiales in the gut microbiota declined in all patients who received piperacillin-tazobactam or meropenem, including patients who did not subsequently develop acute GVHD of the gut or liver (Figure 3). The abundance of genes for butyrate biosynthesis also declined among patients receiving anaerobic antibiotics (P=0.01), from a median (IQR) of 203 (63, 514) reads per kilobase million (RPKM) prior to antibiotics to 0 (0, 10) RPKM during treatment with anaerobic antibiotics. In contrast, the median (IQR) RPKM of butyrate biosynthesis genes did not differ in samples collected before and during treatment with cefepime [151 (40, 351) vs. 62 (27, 209); P=0.22]. Among patients receiving anaerobic antibiotics or cefepime, the abundance of genes for butyrate biosynthesis was strongly correlated with subsequent onset of acute gut/liver GVHD. Specifically, patients who later developed acute gut/liver GVHD had higher abundances of butyrate biosynthesis genes prior to antibiotics [median (IQR) RPKM: 512 (319, 799) vs. 94 (37, 151); P<0.0001] and a more substantial decline in butyrate biosynthesis gene
abundance with antibiotic treatment [median (IQR) RPKM: 512 (319, 799) to 29 (3, 64); \(P=0.0001\)].

**DISCUSSION**

In this study of children and adults with febrile neutropenia after allogeneic HSCT, we found that receipt of piperacillin-tazobactam or a carbapenem increased the risk of acute GVHD of the gut or liver and acute GVHD mortality. In a small group of pediatric patients in this study, anaerobic antibiotics were associated with a loss of bacterial genes for butyrate biosynthesis from the gut metagenome, a finding that preceded onset of acute gut/liver GVHD.

Our findings are consistent with several recent studies that suggest that use of antibiotics with an anaerobic spectrum of activity increases GVHD risk after allogeneic HSCT. Shono et al. reported that empirical treatment of febrile neutropenia with piperacillin-tazobactam or imipenem-cilastatin was associated with a higher risk of combined (acute or chronic) GVHD mortality than aztreonam or cefepime among 383 adult allogeneic HSCT recipients.\(^6\) Using a murine model of allogeneic HSCT, the authors demonstrated that treatment with piperacillin-tazobactam or imipenem-cilastatin was associated with loss of Clostridiales from the gut microbiota while treatment with aztreonam or cefepime generally spared this bacterial order.\(^6\) In 211 adults who underwent allogeneic HSCT, receipt of carbapenem antibiotics (compared with no antibiotics) during the neutropenic period was associated with the development of acute gut GVHD.\(^7\) Finally, in a study of 15 pediatric allogeneic HSCT recipients at two institutions, the number of days of anaerobic antibiotics received for febrile neutropenia was associated with acute GVHD risk.\(^8\) Notably, these studies had small sample sizes, did not directly compare anaerobic antibiotics to other broad-spectrum antibiotic regimens, or did not account for key confounding factors such as GVHD prophylaxis regimen. Our study overcomes these limitations in demonstrating that use of anaerobic antibiotics for febrile neutropenia after allogeneic HSCT is specifically associated with the development of acute GVHD of the gut or liver. This finding was observed in both pediatric and adult allogeneic HSCT recipients and despite differing use of antibiotic prophylaxis in these populations. In contrast, exposure to anaerobic antibiotics was not associated with chronic GVHD in our cohort. Although relatively little is known about the pathophysiology of chronic GVHD, B cells are believed to play a prominent role and it is possible that the gut microbiome has less influence on the number and function of these cells.\(^21\)

Commensal gut anaerobes play an important role in maintaining intestinal homeostasis, and a number of recent studies suggest that these bacteria may modify the risk of GVHD. In particular, a reduction in bacterial diversity and losses of obligate anaerobic bacteria from the order Clostridiales have previously been associated with the risk of GVHD.\(^8\),\(^9\),\(^22\)–\(^24\) Clostridiales prevent inflammation and promote immune tolerance in the gut through the production of butyrate, which induces the differentiation of intestinal regulatory T cells.\(^10\),\(^25\),\(^26\) Regulatory T cells are potent suppressors of immune responses and are believed to play an important role in mitigating the severity of GVHD after allogeneic HSCT.\(^27\),\(^28\) Loss of intestinal butyrate after allogeneic HSCT was previously demonstrated with exposure to anaerobic antibiotics and was associated with the risk of development of GVHD in a study.
of 42 pediatric HSCT recipients.\textsuperscript{29} Moreover, butyrate was reduced in the intestinal tissue of mice after allogeneic HSCT, and the administration of butyrate to these mice via intragastric gavage lowered GVHD clinical scores and improved survival.\textsuperscript{10} In contrast, intestinal butyrate levels at the time of conditioning and two weeks after HSCT were not associated with acute GVHD in a study of 50 adult allogeneic HSCT recipients.\textsuperscript{30} Our findings are broadly consistent with these prior studies while indicating that the magnitude of the change in intestinal butyrate levels resulting from broad-spectrum antibiotics may be more important than the absolute level. In addition, in our cohort, anaerobic antibiotic treatment was associated with a reduction in the relative abundance of \textit{Bifidobacterium} in the gut microbiota. Although bifidobacteria do not directly produce butyrate, their role in promoting growth of Clostridiales has previously been described in detail.\textsuperscript{31,32}

Our results have several important implications for the care of patients undergoing allogeneic HSCT. First, we present data from the largest cohort to date demonstrating that use of anaerobic antibiotics for febrile neutropenia increases acute GVHD risk and mortality in pediatric and adult allogeneic HSCT recipients. Our findings, when combined with previous data from other institutions, indicate that use of piperacillin-tazobactam and carbapenems should be reserved for cases in which anaerobic or multidrug-resistant infection is suspected. In addition, this study demonstrates that measurement of the bacterial genes that encode butyrate biosynthesis enzymes through shotgun metagenomics may provide useful information for assessing risk of patients for acute GVHD of the gut or liver. Although methods such as high-performance liquid chromatography enable direct measurement of butyrate in stool, these approaches are labor-intensive, expensive, and unlikely to be feasible in routine clinical practice.\textsuperscript{33,34} Finally, we found that patients who developed acute gut/liver GVHD had larger declines in the abundance of butyrate biosynthesis genes in the gut metagenome with exposure to broad-spectrum antibiotics. Dramatic shifts in the diversity and composition of the gut microbiota occur after allogeneic HSCT and it is likely that serial monitoring of the gut metagenome and metabolome will be necessary to accurately forecast GVHD and other outcomes after allogeneic HSCT.\textsuperscript{35,36}

This study has several limitations. First, it was conducted at a single academic hospital, the transplant practices of which may differ from those at other transplant centers. Moreover, given the observational study design, patients were not randomly assigned to antibiotic treatments and the potential for unmeasured confounding by indication exists. In addition, although GVHD diagnoses were made by the treating physicians using standardized criteria, this process remains somewhat subjective. We did not record medications administered for the treatment of GVHD, although initial therapy with glucocorticoids was standard throughout the study period at our institution. We reviewed autopsy reports, provider notes, and the results of microbiological testing for all deceased subjects, but misclassification of the cause of death remains possible. For consistency across the study period, we determined degree of HLA matching based only on allele-level typing of HLA-A, -B, and -DRB1. Typing of HLA-C and -DQB1 is currently being performed at our institution and many other transplant centers. Metagenomic analyses were limited to the small number of pediatric patients for whom paired fecal samples were available. Prior studies indicate that the composition of the gut microbiome of healthy children differs from that of healthy adults.\textsuperscript{34} Finally, we used detection of butyrate biosynthesis genes as a surrogate marker for the
functional capacity of the microbiota to produce butyrate. There are few available data to suggest that these gene abundances correlate closely with actual butyrate concentrations in the gut. Moreover, recent studies suggest that other host or gut microbiome-derived metabolites may be associated with onset of acute or chronic GVHD after allogeneic HSCT, including bile acids and branched-chain amino acids.\textsuperscript{37,38}

In summary, we found that the use of anaerobic antibiotics for the empirical treatment of febrile neutropenia was associated with increased risks of acute GVHD of the gut or liver and acute GVHD mortality in adult and pediatric HSCT recipients. Moreover, we demonstrate that exposure to anaerobic antibiotics is associated with loss of genes for butyrate biosynthesis from the gut metagenome, which preceded the onset of acute gut/liver GVHD in our cohort. Our findings indicate that limiting the use of piperacillin-tazobactam and carbapenems to clinical scenarios in which anaerobic or multidrug-resistant infections are suspected could lower GVHD-related mortality in allogeneic HSCT recipients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by research grants from the Derfner Foundation and the Children’s Miracle Network. In addition, this research was supported in part by the National Institute of Allergy and Infectious Diseases (K23AI135090, UM1AI104681) and the National Center for Advancing Translational Sciences (5KL2TR001115, UL1TR002555). LPS was supported by the Agency for Healthcare Research and Quality (5T32HS000032) and the National Cancer Institute (5T32CA116339). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abbreviations:

- CI: confidence interval
- GVHD: graft-versus-host disease
- HLA: human leukocyte antigen
- HR: hazard ratio
- HSCT: hematopoietic stem cell transplantation
- IQR: interquartile range
- RPKM: reads per kilobase million

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HIGHLIGHTS

- Anaerobic antibiotics are associated with an increased risk of acute gut/liver GVHD.
- Piperacillin-tazobactam and carbapenems deplete key gut anaerobes (Clostridiales).
- Loss of butyrate biosynthesis genes from the gut precedes acute gut/liver GVHD.
Figure 1. Cumulative incidence curves for GVHD outcomes by antibiotic group. Cumulative incidence curves are shown for (a) acute gut/liver GVHD and (b) acute GVHD mortality. In multivariable Cox proportional hazards models, the hazards of acute gut/liver GVHD ($P=0.02$) and acute GVHD mortality ($P=0.02$) differed by antibiotic group.
Figure 2. Relative abundances of bacterial orders in fecal samples collected from children before and during antibiotic treatment.
Bar plots depicting the mean relative abundances of bacterial orders in fecal samples collected from 36 children and adolescents within the 10 days prior to initiation of antibiotics (Before Antibiotics) while on antibiotic therapy for at least 48 hours (During Antibiotics). The relative abundances of Bifidobacteriales ($P=0.01$) and Clostridiales ($P=0.002$) declined in children receiving anaerobic antibiotics.
Figure 3. Changes in the abundances of Clostridiales and genes for butyrate biosynthesis among children during antibiotic treatment.

Relative abundances of the bacterial order Clostridiales (a) and genes for butyrate biosynthesis (b), measured as reads per kilobase million (RPKM), are shown for patients before and during treatment with cefepime or anaerobic antibiotics. Each point represents the relative abundance of a single fecal sample and lines are drawn between fecal samples collected within the 10 days prior to initiation of antibiotics (Before Antibiotics) and fecal samples collected while on antibiotic therapy for at least 48 hours (During Antibiotics) for each patient. Samples from patients who subsequently developed acute gut/liver GVHD are shown with a dashed line, while samples from patients who did not develop acute gut/liver GVHD are shown with a solid line. The relative abundance of Clostridiales in the gut microbiome ($P=0.002$) and the abundance of genes for butyrate biosynthesis ($P=0.01$) declined among children receiving anaerobic antibiotics. Butyrate biosynthesis gene abundance also declined markedly during antibiotic treatment among children who later developed acute gut/liver GVHD ($P=0.0001$).
Table 1.
Characteristics of the study population

|                      | Aztreonam, Cefepime, or Ceftazidime (N=723) | Anaerobic Antibiotics (N=491) | P    |
|----------------------|---------------------------------------------|-------------------------------|------|
|                      | N (or median) % (or IQR)                    | N (or median) % (or IQR)      |      |
| Age, years           | 14.4 (5.1, 45.1) 37.4 (15.6, 15.1)         |                               | <0.0001 |
| Sex                  |                                             | 0.29                          |      |
| Female               | 287 40%                                    | 210 43%                       |      |
| Male                 | 436 60%                                    | 281 57%                       |      |
| Race                 |                                             | 0.66                          |      |
| Non-Hispanic white   | 538 74%                                    | 356 72%                       |      |
| Black or African-American | 108 15%                        | 90 18%                        |      |
| Latino or Hispanic-American | 23 3%                                | 18 4%                        |      |
| Other                | 54 7%                                      | 27 5%                         |      |
| Hematological malignancy | 451 62%                        | 388 79%                       | <0.0001 |
| Related HSCT donor   | 243 34%                                    | 139 28%                       | 0.051 |
| HSCT source          |                                             |                               | <0.0001 |
| Peripheral blood     | 218 30%                                    | 215 44%                       |      |
| Bone marrow          | 159 22%                                    | 75 15%                        |      |
| Umbilical cord blood | 346 48%                                    | 201 41%                       |      |
| HLA matching         |                                             | 0.03                          |      |
| 6/6                  | 377 52%                                    | 267 54%                       |      |
| 5/6                  | 155 21%                                    | 76 15%                        |      |
| 4/6 or lower         | 191 27%                                    | 148 30%                       |      |
| Conditioning regimen |                                             | 0.59                          |      |
| Myeloablative        | 570 79%                                    | 381 77%                       |      |
| Non-myeloablative or reduced-intensity | 153 21%                                | 110 22%                       |      |
| GVHD prophylaxis regimen |                                             |                               | 0.01 |
| Calcineurin inhibitor plus methotrexate | 226 31%                                    | 165 34%                       |      |
| Calcineurin inhibitor plus MMF | 344 48%                                    | 202 41%                       |      |
| Receipt of alemtuzumab or ATG | 46 6%                                      | 55 11%                        |      |
| Other                | 107 15%                                    | 69 14%                        |      |

IQR, interquartile range; HSCT, hematopoietic stem cell transplantation; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil; ATG, anti-thymocyte globulin
Table 2.

Antibiotic regimens for febrile neutropenia

|                                | N   | %   |
|--------------------------------|-----|-----|
| Aztreonam/cefepime/ceftazidime group | 723 | 60% |
| Received cefepime or ceftazidime only | 684 | 56% |
| Received aztreonam only          | 39  | 3%  |
| Anaerobic antibiotic group       | 491 | 40% |
| Received piperacillin-tazobactam only | 216 | 18% |
| Received a carbapenem only       | 198 | 16% |
| Received both piperacillin-tazobactam and a carbapenem | 77  | 6%  |
Table 3.

GVHD outcomes by antibiotic group

|                          | Aztreonam, Cefepime, or Ceftazidime (N=724) | Anaerobic Antibiotics (N=492) | Adjusted HR (95% CI) | P    |
|--------------------------|-----------------------------------------------|--------------------------------|----------------------|------|
| Primary outcome          |                                               |                                |                      |      |
| Acute gut/liver GVHD     | 227 31%                                       | 204 41%                        | 1.26 (1.03–1.54)     | 0.02 |
| Secondary outcomes       |                                               |                                |                      |      |
| Acute skin GVHD          | 368 51%                                       | 248 51%                        | 1.05 (0.89–1.25)     | 0.55 |
| 1-year acute GVHD mortality | 45 6%                                      | 55 11%                        | 1.63 (1.08–2.46)     | 0.02 |
| Chronic GVHD             | 264 36%                                       | 159 32%                        | 1.04 (0.84–1.28)     | 0.73 |
| 5-year chronic GVHD mortality | 47 7%                                     | 25 5%                         | 0.88 (0.53–1.45)     | 0.61 |

HR, hazard ratio; CI, confidence interval; GVHD, graft-versus-host-disease
Table 4.
Effect of anaerobic antibiotics on the relative abundances of frequently occurring bacterial genera from the orders Bifidobacteriales and Clostridiales

|                      | Median (Interquartile Range) Relative Abundance |  
|----------------------|-----------------------------------------------|  
|                      | Before Anaerobic Antibiotics | During Anaerobic Antibiotics |  
|                      | **P** |  
| Bifidobacteriales    |  
| Bifidobacterium      | 9.7% (0.06%, 24.3%) | 0% (0%, 0%) | 0.02  
| Clostridales          |  
| Blautia               | 4.1% (0.05%, 9.1%) | 0% (0%, 0%) | 0.01  
| Clostridium           | 1.3% (0.02%, 6.7%) | 0% (0%, 0%) | 0.02  
| Faecalibacterium      | 0% (0%, 2.4%) | 0% (0%, 0%) | 0.18  
| Lachnoclostridium     | 0.2% (0.03%, 1.5%) | 0% (0%, 0%) | 0.01  
| Roseburia             | 0.002% (0%, 0.2%) | 0% (0%, 0%) | 0.06  
| Ruthenibacterium      | 0.09% (0%, 0.6%) | 0% (0%, 0%) | 0.06  

*Bio Blood Marrow Transplant. Author manuscript; available in PMC 2021 November 01.*