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Rifaximin for Preventing Acute Graft Versus Host Disease: Impact on Plasma Markers of Inflammation and T Cell Activation

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Abstract

Rifaximin for Preventing Acute Graft Versus Host Disease: Impact on Plasma Markers of Inflammation and T Cell Activation

By Muna Qayed

Bacterial translocation across damaged gut mucosa is critical in the pathogenesis of acute graft versus host disease (AGVHD). We conducted a pilot trial to test the hypothesis that rifaximin would abrogate systemic inflammation and resultant T cell activation in allogeneic transplant recipients. Twenty adult and pediatric (≥12 years) patients were enrolled. Rifaximin (400 mg bid) was started on day -10 and continued through day +30. Plasma samples were collected at baseline, day 0 (pre-transplant) and day 15 to measure levels of markers of inflammation (soluble TNF receptor 1 [sTNFR1] and interleukin 6 [IL-6]), and donor T cell activation (soluble IL-2 receptor [sIL-2R]). A historical control group (n=24) was formed from subjects enrolled on a previously conducted study. The median percentage of rifaximin doses successfully administered was 95%. There were no serious adverse events attributed to rifaximin. Mean IL-6 concentration decreased by 64% in the treatment group relative to the control group by day 0 (p=0.002). sTNFR1 and sIL-2R did not change in the treatment group relative to the control group. In multivariate analysis, the odds ratio for developing serious bacterial infection for rifaximin was 0.44 (95% CI 0.1, 1.9). This pilot study demonstrates that administering rifaximin to prevent AGVHD is safe and feasible. Rifaximin may limit inflammation as suggested by its effect on IL-6 levels, but its anti-inflammatory effect may be insufficient to prevent downstream activation of donor T cells. The role of rifaximin for infection prophylaxis needs to be investigated in a large scale randomized trial.

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Introduction

Acute graft versus host disease (AGVHD) is a frequent and life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT) (1). The bacterial flora of the gut plays an important role in its pathogenesis. Injury to the intestinal mucosa by pre-transplant conditioning (high dose chemotherapy and radiation) results in bacterial translocation across the intestinal wall. This damage leads to release of a cascade of inflammatory cytokines, which, in turn, amplifies the graft versus host immune response by activating donor T cells, resulting in end organ damage. A corollary to the critical part gut bacteria plays in AGVHD pathogenesis is that oral antibiotic therapy can prevent AGVHD, as suggested by several pre-clinical studies. Rifaximin is a broad spectrum, minimally absorbed oral antibiotic that has been shown to be effective in inflammatory bowel disease. Those features, among several others, suggest that it could be effective for preventing AGVHD. Also, since patients undergoing stem cell transplantation are severely immunocompromised and are at high risk for life threatening infection, rifaximin may have a role in infection prophylaxis. As the first step towards realizing our long range goal of developing more effective antibiotic prophylaxis against AGVHD, we conducted a pilot trial using rifaximin in adult and pediatric HSCT recipients. The primary aims of this trial were to investigate the feasibility of using rifaximin in the immediate post transplant period, and to estimate the effect of rifaximin on plasma levels of biomarkers of inflammation and T-cell activation. We enrolled twenty patients on a single treatment arm, and used a historical control group for comparison. Rifaximin was administered twice daily starting ten days prior to transplant

and continued for six weeks. Biomarker levels were measures at baseline prior to the start of conditioning, at day 0 prior to stem cell infusion, and at day 15 post stem cell infusion. A repeated measures linear mixed effects model was used to analyze biomarker results. Serious bacterial infections were recorded for the time period of rifaximin administration and an estimate odds ratio was calculated using a multivariate logistic regression model. This is the first trial to investigate the effect of gut decontamination on biomarkers of inflammation and T cell activation in HSCT recipients, and if the results are promising, they will be used to help design a large scale clinical trial using gut decontamination for AGVHD prophylaxis.

Background

Allogeneic hematopoietic stem cell transplantation is an important therapy for many malignant and non malignant disorders. AGVHD is the major toxicity and remains a lethal complication of HSCT, limiting its wider application (1). It is driven by donor T cells as they react against disparate host antigens. AGVHD is responsible for 15-40% of transplant related mortality and is a major cause of morbidity (2). It primarily affects three target organs: the gastrointestinal tract, the liver and the skin.

The role of gut flora in the pathophysiology of acute graft versus host disease

The development of AGVHD can be conceptualized in three phases. The earliest phase is initiated by the damage caused by the HSCT conditioning regimens, including total body irradiation and chemotherapy (phase I, recipient conditioning). This leads to the secretion of proinflammatory cytokines (such as tumor necrosis factor α, interleukin-1 and interleukin-6). Importantly, injury to the gastrointestinal (GI) tract results in translocation of bacteria and bacterial products (particularly lipopolysaccharide) across the intestinal wall. These further stimulate host antigen presenting cells (APCs), and activate macrophages, which in turn further produce pro-inflammatory cytokines. The second phase is initiated when donor T cells are infused into the host (phase II, donor T cell activation). This includes antigen presentation by activated APCs, donor T cell activation, proliferation, and migration into target tissues. Target organ damage follows as a result of multiple cellular and inflammatory cascades involving cytotoxic T-lymphocytes and inflammatory cytokines (phase III, inflammatory effectors).

Gastrointestinal injury also primes the inflammatory effector cells that are subsequently recruited by donor T cells (3, 4). Thus, the gut flora plays an integral role in the development of AGVHD.

Serum biomarkers in AGVHD

Plasma levels of interleukin-6 (IL-6), soluble tumor necrosis factor receptor-1 (sTNFR1) and soluble interleukin-2 receptor (sIL-2R) were measured serially in this study and were utilized to study the effect of rifaximin on tissue inflammation and donor T cell activation. Plasma IL-6 level is a marker of inflammation, and plays a pivotal role in directing the immune response toward an inflammatory phenotype and away from a regulatory response (5). Levels of tumor necrosis factor (TNF-α) rise post-transplant, peaking about a week post-transplant and before the onset of GVHD, presumably, in part, from the tissue inflammation caused by bacterial translocation (6, 7). TNF-α is difficult to measure directly because it often circulates bound to its receptor, but sTNFR1 is a good surrogate marker for TNF- α (8). Levels of sIL-2R, a marker of T cell activation, also increase post-transplant, peaking during the third week post-transplant concordant with the onset of GVHD, and the level correlates with GVHD severity (6).

Management of AGVHD and the rationale for gut decontamination

Prevention is the mainstay of GVHD management. Most patients receive calcineurin inhibitor (cyclosporine or tacrolimus) based prophylaxis post-transplant (9). Despite this, moderate to severe (grade II-IV) GVHD develops in 30% or more of HLA matched related transplants and an even greater percentage of unrelated donor

transplants. Corticosteroids have been the primary therapy for AGVHD for more than three decades (10). This therapy is effective in only about 50 to 60% of cases, and usually fails in severe cases, making severe AGVHD fatal more than half the time (11). There is no standard effective treatment available for steroid refractory AGVHD, and attempts at augmenting initial therapy by combining corticosteroids with other agents have been ineffective (12).

These attempts have largely focused on inhibiting donor T cells. A different approach to AGVHD prophylaxis that has not been well studied is to counteract the pro-inflammatory effects of the intestinal flora through gut decontamination. Pre-clinical data dating back to the 1970's showed that mice receiving allogeneic bone marrow after high dose total body irradiation could be protected from GVHD by raising them in germ free conditions or by "decontaminating" the gut with non-absorbable anti-bacterial antibiotics (13, 14). In different murine studies, countering the effect of the gut flora, using an endotoxin antagonist, diminished the incidence of GVHD (15).

In the clinical setting, the introduction of a "protective environment'' improved survival, and to a lesser extent decreased, or delayed, the onset of AGVHD. The protective environment included gut decontamination with various combinations of vancomycin, gentamicin, polymyxin B, and others, a low microbial diet, aggressive skin cleansing, and isolation in a laminar air flow room (16, 17). A retrospective review of matched sibling donor transplants suggested that anaerobic bacterial growth suppression may modulate the occurrence of moderate to severe AGVHD, and another report suggested that gram-positive bacteria maybe relevant to promoting inflammation (18). In the only reported randomized controlled clinical trial to assess the efficacy of gut

decontamination for AGVHD prophylaxis, oral metronidazole (in combination with ciprofloxacin) was shown to reduce the incidence of grade II-IV GVHD (25%), when compared to ciprofloxacin alone (50%). These results were only statistically significant in matched related donor transplants, which constituted the majority of the study population (19).

The rationale for using rifaximin for preventing AGVHD

Rifaximin, a non-absorbable rifamycin derivative, has several features that give it a theoretical advantage over other antibiotics for gut decontamination. It is a bacteriostatic and thus may be less likely to induce release of endotoxin when compared to bactericidal antibiotics (20, 21, 22). It has broad spectrum activity, covering both gram-positive and gram-negative bacteria and aerobes as well as anaerobes (23, 24). This is in contrast to other agents that have been used to prevent GVHD, such as polymixin B (gram negative aerobes) and metronidazole (anaerobes). Rifaximin is clinically effective for managing several gastrointestinal disorders, including preventing travelers' diarrhea and treating hepatic encephalopathy and small intestinal bacterial overgrowth (25, 26, 27, 28). Furthermore, in a murine colitis model, rifaximin inhibited bacterial translocation and diminished the secretion of inflammatory cytokines (29). Rifaximin has an excellent safety profile, and since it is non-absorbable, has minimal drug-drug interactions (25).

The dosing recommendations for rifaximin depend on the indication for use, and range from 600 to 1200 mg daily (Micromedex). It is FDA approved for adults and children ages 12 and older. In our pilot trial, adults and children ages 12 and over who

weighed at least 40 kg were eligible for enrollment, and a daily dose of 800 mg (divided twice daily) was used.

Rifaximin for prophylaxis against infection

Bacterial infections are a major cause of complications and death in patients with hematologic malignancies and chemotherapy-induced neutropenia. Various approaches have been tried over the past few decades for prophylaxis against bacterial infections in neutropenic patients. In a recent double-blind, randomized, placebo-controlled trial utilizing levofloxacin in adult patients with chemotherapy-induced neutropenia, statistically significant reductions were seen in the levofloxacin arm with regard to fever, microbiologically documented infections, bacteremia, and single-agent gram-negative bacteremia. There was no change in mortality (30). Two recent meta-analyses that evaluated antibiotic prophylaxis for neutropenic patients confirmed the above findings and found statistically significant reductions in overall mortality (31) and infection related mortality (32). Despite these potential advantages, routine use of prophylactic agents for neutropenia prophylaxis remains controversial, mainly because of the unknown long-term consequences on antimicrobial resistance and flora (33).

However, much of the experience with prophylaxis in neutropenia has been with fluoroquinolones, which are known to induce resistance and alter gut flora. Drugs that are less apt to induce resistance and perturb the flora, such as Rifaximin, may represent an effective alternative. Collectively, non-absorbable antibiotics, such as fluoroquinolones, have been shown to prevent infection and improve survival in

neutropenic patients (31). Rifaximin, with its broad spectrum of activity, might therefore prove to be a particularly useful prophylactic agent.

Methods

Null Hypothesis

Primary aim:

The proportion of patients who are successfully administered ≥ 75% of the scheduled rifaximin doses is less than 80%.

The change in mean levels of biomarkers of inflammation and T-cell activation in the Rifaximin treatment group is equal to that in the control group, controlling for other covariates.

Secondary aim:

The proportion of patients who develop serious bacterial infection in the Rifaximin treatment group is equal to that in the control group controlling for other covariates.

Specific Aims:

The primary aim of this study was to 1) investigate the feasibility and compliance of using Rifaximin in the immediate post transplant period; 2) estimate the effect of rifaximin on plasma levels of biomarkers of inflammation (IL-6 and sTNFR1) and T-cell activation (sIL-2R).

The secondary aim of this study was to obtain preliminary data on the efficacy of administering rifaximin for prophylaxis against serious bacterial infections in HSCT patients.

Study Design

Pilot clinical trial with a single treatment arm and a historical control arm.

Participants:

Subjects were recruited through the Children's Healthcare of Atlanta Blood and Marrow Transplant Program and the Winship Cancer Institute Adult Blood and Marrow Transplant Program.

Inclusion Criteria

1. Patients 12 years of age or older.
2. Patients were eligible regardless of their type of disease (malignant or non-malignant), type of donor (HLA matched related, mismatched related or unrelated donors), type of hematopoietic cell source (unstimulated marrow, cytokine stimulated marrow, cytokine stimulated peripheral blood or umbilical cord blood), or GVHD prophylaxis.
3. Patients receiving a myeloablative or moderately intensive reduced intensity conditioning regimen (at least 8 mg/kg oral busulfan or the equivalent IV dose, or at least 100 mg/m^2 of melphalan , or at least 100 mg/kg of cyclophosphamide, or at least 500 cGy of TBI).

Exclusion Criteria

1. Known hypersensitivity to rifaximin or other rifamycin antimicrobial agents.

2. Minimally toxic conditioning regimen (e.g., low dose TBI based), since these regimens induce minimal myelosuppression and gut injury.
3. Patients with documented severe active infection (viral, bacterial, fungal, and protozoal) were not eligible. Patients with uncomplicated fevers, minor infections (such as catheter site infection, presumed bacterial sinusitis or viral URIs (negative for parainfluenza, influenza, RSV and adenovirus)) and simple bacteremia (not complicated by signs of shock or associated with difficult to treat sites of infection (e.g., typhilitis, peri-rectal cellulites or abscess, pneumonia)) were eligible. These patients were excluded primarily because of the potential confounding effect of infection on biomarker levels.
4. Patients with treatment unresponsive hematologic malignant diseases (based on an assessment done within two weeks of the start of conditioning therapy). These patients were excluded primarily because of the potential confounding effect of active malignancy on the biomarker levels.
    i. Acute leukemia with greater than 20% blasts and/or grossly detectable extramedullary disease at the time of transplant.
    ii. Lymphomas with less than a partial response .
5. Following the standard practice in HSCT, pregnant or breast feeding patients were excluded.

Historical Controls:

The historical control group was formed out of participants in the pilot prospective cohort study 'Plasma markers of T cell activation as biomarkers for acute

graft versus host disease' and who fulfilled enrollment criteria for the rifaximin study. This study was conducted between May 2006 and May 2008 and accrued 61 participants; 30 patients met our inclusion criteria. Since all the participants in the rifaximin pilot study had a primary oncologic diagnosis, six patients with a primary hematologic diagnosis were further excluded from the control group, leaving 24 historical controls. The controls had the same data, clinical outcomes, and blood samples collected at similar time points as the treatment group. Data on plasma sIL-2R and sTNFR1 levels in the controls were available for analysis, and banked plasma samples were used to measure IL-6 levels.

Clinical procedures and data collection:

The study treatment was started on day -10 and continued through day +30 (Day 0 was designated as the day of stem cell infusion). For patients who weighed over 40 kg the dose was 400 mg (two tablets) every 12 hours. All other aspects of therapy, including conditioning, donor and source of hematopoietic cell source selection, post-transplant immune suppression, and supportive care were left to the discretion of the treating physician. The use of prophylactic antibacterial antibiotics other than Rifaximin was permitted.

Clinical assessment for toxicities, adverse effects, development of GVHD and all bacterial, fungal and viral infections was performed and recorded weekly through day +30, and then at day +60 and day +100. Adherence to study treatment, and the number of intended rifaximin doses that were successfully administered were recorded weekly while participants were inpatient, and by self report/ patient calendar after discharge.

Blood samples for biomarker assays were collected at the following time-points: prior to start of conditioning (anytime during the preceding 7 days), day of transplant prior to cell infusion, then on days +5 (+/- 1 day), +10(+/- 1 day), +15(+/- 1 day), +30(+/- 3 days), and +45 (+/-3 days). Samples were analyzed for IL-6, sTNFR1 and sIL-2R by sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Bender Medsystems, Vienna, Austria and R&D Systems, Minneapolis, MN).

Study Definitions:

Feasibility: Rifaximin administration in the peritransplant period if at least 75% of scheduled doses were successfully administered in at least 80% of patients.

Acute GVHD: Acute GVHD was graded using established criteria(11). Patients were considered not evaluable for acute GVHD if they died before day 30 and had not developed acute GVHD.

Toxicities: Organ toxicities were recorded using the Common Terminology Criteria for Adverse Events v3.0 of the National Cancer Institute.

Serious bacterial infections: The occurrence of bacteremia/line sepsis, central venous line site/tunnel infection, peri-rectal infection, typhlitis/neutropenic enterocolitis, pneumonia, Clostridium difficile colitis.

Sample Size:

The purpose of this pilot study was to obtain estimates of mean biomarker levels and variances, as well as other preliminary data needed to calculate the sample size requirements for a full scale study.

Statistical Analysis:

To test the feasibility of administering rifaximin to transplant patients, the binomial test of significance was used to test the probability that the proportion of patients who had ≥75% of the scheduled doses administered successfully was ≥80%. The treatment and historical control groups were assessed for differences in baseline characteristics using the two sample t-test and Wilcoxon rank sum test for continuous variables and χ^2 test for categorical variables. The measurement reliability of the biomarker assays was assessed with the intra-class correlation coefficient. Biomarker variables were not normally disturbed and were log transformed prior to statistical testing. The lower detectable limit for IL-6 levels was 0.74 pg/ml; patients with an undetectable level of IL-6 at baseline (0-0.74) were assigned a value of 0.37 prior to transformation. Mean Biomarker levels concentrations were calculated for each group at baseline, day 0, and day 15 for IL-6 and sTNFR1, and at baseline and day 15 for sIL-2R. Treatment effects were evaluated by assessing the differences in biomarker concentrations from baseline to follow-up between the treatment and historical control group by repeated measures linear mixed effects model, as implemented using the Proc Mixed procedure in SAS. The model included the intercept, treatment, and a treatment x visit interaction term with an unstructured covariance matrix. Kenward-Roger's adjusted degrees of freedom was used, an approach specifically designed for small sample settings (34). Absolute treatment effects were calculated as the absolute change from baseline in the treatment group minus the absolute change from baseline in the control group. Since concentrations of the measured biomarkers in plasma are not widely familiar in clinical

practice, to provide perspective on the magnitude of treatment effects, relative effects were calculated, defined as (treatment group follow-up/treatment group baseline)/(control group follow/up/control group baseline). The relative effect provides an estimate of the proportional change in the treatment group relative to that in the control group. The interpretation of the relative effect is somewhat analogous to that of an odds ratio. A multivariate analysis was performed to control for the imbalance of baseline covariates in the two groups. The effect of rifaximin on the development of bacteremia and serious bacterial infection was assessed using multivariate logistic regression, adjusting for baseline covariates. Analysis was performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC, USA). A cutoff level of $p \leq 0.05$ (two-sided) was used for assessing statistical significance.

Human Subjects Protection:

The study was approved by the Emory and Children's Healthcare of Atlanta institutional review boards (IRB). Informed consent was obtained by one of the co-investigators using forms that were reviewed and approved by the Emory and Children's Healthcare of Atlanta IRBs, following the guidelines on the use of human subjects in research. Written assent was obtained from children ages 12–17 years, using an approved informed assent statement, as well as parent or guardian informed consent.

A waiver of the Investigational New Drug Application (IND) was obtained from the FDA prior to enrollment. An internal data and safety monitoring board (DSMB) was formed, consisting of three non-transplant hematology-oncology providers from Emory University and Children's Healthcare of Atlanta, and data regarding adverse events,

engraftment, acute graft versus host disease, regimen related toxicity, and infection was provided for interim analysis.

Results

Study participants

Baseline characteristics of the study participants and historical controls are shown in Table 1. The treatment group was significantly younger than the control group (median age 17.3 years compared to 38.8 years). There was also a significant difference in donor type and source of stem cells. The treatment group had a significantly higher proportion of patients undergoing a mismatched unrelated donor transplant, including double cord transplants while the control group had only one patient (4.2%) who underwent a mismatched unrelated transplant, and no cord transplants. All patients had a primary diagnosis of a malignancy and there was no significant difference in the subtypes of malignancies between the two groups. All patients received a myeloablative or moderate intensity preparative regimen, and there was no significant difference in the proportion of patients who received a total body irradiation (TBI) based regimen between groups.

Feasibility and adherence

Rifaximin was administered in tablet form by mouth or in crushed form via nasogastric tube twice daily for 41 days (Days -10 through day +30). Because the effect of rifaximin may be more important early in the transplant process, we analyzed adherence in two time periods; the first 21 days of administration (day -10 through day +10), and the complete period of administration (day -10 through day +30). One patient was started on rifaximin on the day of transplant due to a scheduling error, and was

included in the analysis. Two patients were excluded from the analysis of the complete time period; one patient died before day +30, and for the second patient there was inadequate documentation of outpatient administration once that patient was discharged (after day +10). The median proportion of successfully administered doses for both time periods was 95% (Table 2). The only patient who received <75% of the scheduled doses in the first 21 days was the patient who was started on day 0 due to scheduling errors. The main reasons for inability to administer the drug were severe mucositis, nausea and vomiting resulting in inability to tolerate oral intake, and acute clinical deterioration precluding any oral intake. An additional factor in younger patients was blockage of nasogastric tubes when administering the crushed form of the drug, necessitating tube replacement. There were no serious adverse events attributed to rifaximin. The most common reported side effects were nausea and vomiting.

Clinical outcomes

The time to engraftment (defined as the first of three consecutive days with an absolute neutrophil count greater than 500 cells/μL) was similar in the two groups. The median number of days to engraftment was 17 days in the treatment group (range 7-38 days), and 16 days in the control group (range 7-23) (Wilcoxon rank sum p-value =0.4). There was one patient who failed to engraft in each group. Those patients were analyzed for infection outcomes, but excluded from the biomarker analysis. The overall survival at day 100 was 84.7% in the treatment group and 87.5% in the control group (log-rank p value= 0.74). The incidence of acute GVHD was similar in the two groups (Table 3).

Effects of rifaximin on IL-6, STNFR1 and sIL-2R concentrations

Measurement reliability assessed by intra-class correlation coefficients were 0.91, 0.94 and 0.90 for IL-6, TNFR1, and sIL-2R, respectively. Table 4 shows the effects of rifaximin on plasma biomarker concentrations relative to the controls. After 10 days of treatment (day -10 to day 0 of transplant, prior to stem cell infusion), mean IL-6 concentration decreased by 71% in the treatment group relative to the control group ($p=0.002$), and by day 15 of transplant mean IL-6 levels decreased by 65% in the treatment group relative to the control group ($p=0.09$). Overall, mean STNFR1 level did not change in the treatment group relative to the control group. sIL-2R level was assessed at baseline and day 15; day 0 levels were not evaluated since sIL-2R is a marker of T-cell activation. By day 15, mean sIL-2R level increased by 67% in the treatment group relative to the control group.

To control for imbalances in baseline characteristics between the two groups, a multivariate analysis was performed. Age was transformed into a categorical variable (with the median age of 29 years used as a cut-off), and donor type and stem cell source were categorized together (matched related vs. alternate donor). Controlling for age and donor type did not appreciably change the results (Table 5).

Frequency of infections

Patients were considered to have an event if they developed bacteremia or serious bacterial infection between days -10 and day 30 of transplant. Due to the short period of follow-up, only the first episode of infection was considered in the analysis for patients who developed multiple infections. The frequency and types of infections are detailed in

Table 6. For the treatment group, the odds ratio for developing bacteremia was 0.79 (95% confidence interval [95% CI] 0.24 – 2.62), and for serious bacterial infection 0.85 (95% CI 0.26 – 2.78). There was no difference in the frequency of Clostridium difficile colitis, and no increase in the incidence of opportunistic fungal infections in the treatment group. A multivariate logistic regression analysis was performed to control for other covariates (Table 7). For the treatment group, the odds ratio for developing bacteremia was 0.28 (95% confidence interval 0.05 – 1.6) and for developing any serious bacterial infection 0.44 (95% confidence interval 0.1 – 1.9). Since all patients older than 18 years of age were on other prophylactic antibiotics, we were not able to control for age and other antibiotic use separately, and thus we were unable to assess for interaction between the use of rifaximin and other prophylaxis.

Discussion

Consistent with murine models in which the role of bacterial translocation across damaged gut mucosa was found to be critical in the pathogenesis of AGVHD, in this pilot trial of gut decontamination in patients undergoing allogeneic stem cell transplantation, we found that prophylactic oral rifaximin administration led to statistically significantly lower serum IL-6 levels, especially within the first two weeks of treatment; however, there was no evidence for treatment effects on sTNFR1 levels or subsequent T cell activation. We also found that, overall, rifaximin administration was safe, and did not result in increased opportunistic infections.

Rifaximin administration in the initial 20 days of transplant was feasible, with nausea being the main reported side effect, although this could also be attributed to concurrent chemotherapy administration in the conditioning phase. Severe nausea, recurrent vomiting, and severe mucositis interfered with the administration of this oral medicine after day 10 of transplant. The occurrence of these symptoms is expected among patients undergoing myeloablative or moderate intensity conditioning regimens. To circumvent this problem, we planned to reconstitute crushed rifaximin tablets for administration via nasogastric tubes. This solution, however, was not feasible in the pediatric population, as it resulted in recurrent blockage of these tubes requiring replacement. The use of rifaximin in pediatric patients where the development of mucositis is anticipated requires an improved formulation, in the form of a suspension, or possibly a paste.

Our biomarker data suggest that rifaximin may abrogate systemic inflammation by inhibiting bacterial translocation as evidenced by its effect on IL-6 levels by day 0. However, it did not result in downstream inhibition of T cell activation. This raises several questions about the role of inflammation in the pathogenesis of GVHD. The Ferrara model for the pathophysiology of GVHD is based largely on murine studies, and has long guided research in this field (35). Our results, which are drawn from a sample comprised predominantly of patients receiving alternative donor transplants (mismatched or unrelated transplants), are consistent with the previously conducted randomized controlled trial, where gut decontamination reduced the incidence of GVHD by 50%, but only in matched sibling donor transplants (19). Taken together, the results of these two studies suggest that the role of the gut flora may not be central in the pathogenesis and clinical development of acute GVHD in settings with large degrees of histoincompatibility.

Rifaximin results in broad spectrum inhibition of bacterial growth. Earlier studies pointed out the importance of gram negative bacteria and the release of lipopolysaccharide in the inflammatory cascade preceding T cell activation (15, 17), and the subsequent randomized trial demonstrated an advantage to the addition of anaerobic coverage (19). There are no pre-clinical data to suggest that some bacterial flora may exert a protective effect on gut inflammation and injury, but broad inhibition of bacteria, as produced by rifaximin, may not be optimal and needs to be studied in murine models.

Because the diagnosis of infection may be subjective, and relies on the evaluating physician to some extent, we evaluated the efficacy of rifaximin for prophylaxis by estimating the effect on serious bacterial infection, in addition to bacteremia, as that is an

objective diagnosis requiring the presence of a positive blood culture. All adult patients received prophylactic antibiotics during their transplant period. The results of our multivariate analyses suggest that the use of rifaximin may protect against bacteremia and serious bacterial infection; however, the confidence interval for the estimated effect was wide as expected from a pilot trial with a small sample size (power at $\alpha = 0.05$ was 55%). To prospectively study the efficacy of rifaximin for prophylaxis against serious bacterial infection in a randomized controlled design, and assuming that serious bacterial infection occurs in approximately 80% of patients undergoing myeloablative stem cell transplantation, we estimate that at least 150 patients (75 in each group) would be needed to detect an absolute difference of at least 20% between rifaximin and placebo with a statistical power of 80% and a 5% significance level.

Our study is one of few reported clinical trials of gut decontamination for GVHD prophylaxis, and is the only trial using rifaximin for this purpose. Furthermore, it is the only trial of the effect of gut decontamination on inflammatory biomarkers in stem cell transplant recipients. The major limitations of this study are the nonrandomized design and the use of historical controls, as the treatment arm and the control arm were not concurrent. The lack of randomization and the heterogeneity of subjects and baseline differences between the two groups - especially in the setting of a small sample size - make it difficult to draw definite conclusions from the results.

In conclusion, our findings raise questions about the importance of the gut flora in the inflammatory cascade that culminates in donor T cell activation and clinical GVHD. Its role might predominantly be in the setting of matched related donor transplants and minimal histoincompatibility. This needs to be verified in animal models, and through

further study of the inflammatory cascade in stem cell transplant recipients. Rifaximin may hold promise as a prophylactic agent against serious bacterial infection in patients undergoing stem cell transplantation or intensive chemotherapy for various malignancies. Using estimates from this study, a prospective randomized large scale trial will be conducted to investigate that potential use.

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Table 1: Baseline characteristics of study participants and historical controls

Characteristic	Treatment Group N=20	Control Group N=24	P-value[1]
Age (years)			
Median	17.3	38.8	0.03
Interquartile range	23.8 (14.4 – 38.2)	31.7 (19.3 – 50.9)	
Sex (%)			
Male	12 (60.0)	16 (66.7)	
Female	8 (40.0)	8 (33.3)	0.65
Disease (%)			
ALL	4 (20.0)	4 (16.7)	
AML	8 (40.0)	13 (54.2)	
MDS	3 (15.0)	5 (20.8)	
Other Leukemia	2 (10.0)	1 (4.2)	
Lymphoma	3(15.0)	1 (4.2)	0.64
Donor (%)			
Matched related	3 (15.0)	8 (33.3)	
Mismatched related	2 (10.0)	3 (12.5)	
Matched unrelated	8 (40.0)	12 (50.0)	
Mismatched unrelated	7 (35.0)	1 (4.2)	0.06
Stem Cell Source (%)			
Peripheral Blood	8 (40.0)	17 (70.8)	
Bone marrow	5 (25.0)	7 (29.2)	
Cord	4 (20.0)	0	
Double cord	3 (15.0)	0	0.01
Conditioning (%)			0.45
TBI based	9 (45.0)	6 (25.0)	
Bu/Cy	5 (25.0)	5 (20.8)	
Mel/Flu	3 (15.0)	7 (29.2)	
Other	3 (15.0)	6 (25.0)	
GVHD Prophylaxis (%)			
CI+MTX	9 (45.0)	14 (58.3)	
CI+MMF	11 (55.0)	9 (37.5)	
Other	0	1 (4.2)	0.36

Abbreviations: ALL acute lymphoblastic leukemia, AML acute myeloid leukemia, MDS myelodysplastic syndrome, TBI total body irradiation, CI calcineurin inhibitor, MTX methotrexate, MMF mycophenolate mofetil.

[1] P-value calculated by Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables, 2-sided p value calculated at alpha=0.05.

Table 2: Feasibility of and adherence to rifaximin administration

Time Period	Median % administered	Patients evaluable	Proportion received >75% of doses	P value[1]
Day $^{-}10 - {}^{+}10$	95%	20	95.0%	0.047
Day $^{-}10 - {}^{+}30$	95%	18[2]	83.3%	0.36

[1]Binomial test (testing proportion ≥ 0.80).
[2] Two patients excluded from analysis due to death before day 30, and lack of documentation of administered doses.

Table 3: Cumulative incidence of clinical GVHD in the treatment and control groups

GVHD	No./Total (Cumulative Incidence) Treatment	Control	P value[1]
Moderate-severe (grade 2-4)	13/18 (69%)	13/23 (58%)	0.63
Severe (grade 3-4)	6/18 (34%)	5/23 (22%)	0.50
Gastrointestinal (stage 1-4)	10/18 (58%)	10/23 (45%)	0.58

[1]Log rank test, 2-sided p value calculated at alpha=0.05.

Table 4: Univariate analysis for effects of rifaximin on biomarkers of inflammation and T cell activation

Biomarker	Control Mean (SE)	Treatment Mean (SE)	Absolute Treatment Effects[1] Mean (95% CI)	Relative Treatment Effects[2]	P value[3]
IL-6 (pg/ml)[4]					
Baseline	1.08 (0.23)	2.43 (0.60)			
Day 0[5]	3.09 (0.74)	2.03 (0.55)	-1.22 (-1.94, -0.49)	0.29	0.002
Day 15	9.94 (3.30)	7.68 (2.69)	-1.06 (-1.95, 0.17)	0.35	0.09
sTNFR1 (pg/ml)					
Baseline	1,811 (176)	1,442 (154)			
Day 0	2,243 (155)	2,097 (163)	0.16 (-0.15, 0.47)	1.17	0.30
Day 15	3,532 (343)	3,197 (272)	0.13 (-0.21, 0.47)	1.15	0.45
sIL2R (ng/ml)					
Baseline	5.35 (0.96)	3.54 (0.67)			
Day 15	12.89 (2.84)	14.24 (3.28)	0.51 (-0.29, 1.31)	1.67	0.20

Abbreviations: IL-6 interleukin-6, sTNFR1 soluble tumor necrosis factor-1, sIL2R soluble interleukin-2 receptor.

[1]Absolute treatment effect is the absolute change from baseline to follow-up in the treatment group minus the absolute change from baseline to follow-up in the control group from the repeated measures linear mixed effects model.

[2]Relative treatment effect is defined as: (treatment group follow-up/treatment group baseline)/(control group follow-up/control group baseline). The interpretation of the relative effect is similar to that of an odds ratio.

[3]P values for baseline to follow-up difference between treatment and control groups from mixed effects model, 2-sided p value calculated at alpha=0.05.

[4]Geometric means with standard errors, calculated by exponentiating the mean of the log transformed values.

[5]Day 0 indicates day of transplant.

Table 5: Multivariate[1] analysis for effects of rifaximin on biomarkers of inflammation and T cell activation

Biomarker	Control Mean (SE)	Treatment Mean (SE)	Absolute Treatment Effects Mean (95% CI)[2]	Relative Treatment Effects[3]	P value[4]
IL-6 (pg/ml)[5]					
Baseline	1.06 (0.22)	2.34 (0.58)			
Day 0[6]	3.01 (0.72)	1.96 (0.57)	-1.01 (-1.95, -0.50)	0.29	0.001
Day 15	9.50 (3.28)	7.46 (2.69)	-0.87 (-2.27, 0.20)	0.35	0.10
sTNFR1 (pg/ml)					
Baseline	1,782 (1,099)	1,528 (1,105)			
Day 0	2,218 (1,062)	2,220 (1,083)	155 (-156, 466)	1.17	0.32
Day 15	3,487 (1,083)	3,372 (1,094)	121 (-220, 462)	1.13	0.48
sIL2R (ng/ml)					
Baseline	5.50 (1.21)	3.54 (1.22)			
Day 15	13.27 (1.25)	13.16 (1.28)	0.53 (-0.27, 1.32)	1.69	0.19

[1]IL-6, sIL2R: controlling for age and donor type (matched related donor or alternate donor), sTNFR1: controlling for age, donor type, and exposure to total body irradiation
[2]Absolute treatment effect is the absolute change from baseline to follow-up in the treatment group minus the absolute change from baseline to follow-up in the control group from the repeated measures linear mixed effects model.
[3]Relative treatment effect is defined as: (treatment group follow-up/treatment group baseline)/(control group follow-up/control group baseline). The interpretation of the relative effect is similar to that of an odds ratio.
[4]P values for baseline to follow-up difference between treatment and control groups from mixed effects model, 2-sided p value calculated at alpha=0.05.
[5]Geometric means with standard errors, calculated by exponentiating the mean of the log transformed values.
[6]Day 0 indicates day of transplant.

Table 6: Frequency and types of infections in the treatment and control groups

Infection	Treatment (N=20)	Controls (N=24)	P value[1]	OR[2] (95% CI)
Bacteremia (%)	8 (40%)	11 (45.8%)	0.70	0.79
Gram positive	5	8		(0.24 – 2.62)
Gram negative	3	2		
Mixed organism	0	1		
Serious Bacterial Infection (%)	10 (50%)	13 (54.2%)	0.78	0.85
Bacteremia	8	11		(0.26 – 2.78)
Clostridium difficile	1	1		
Pneumonia	1	1		
Catheter-related infection	1	0		
Perirectal infection	0	1		

[1]Chi square P value, alpha =0.05.
[2]Crude odds ratio for rifaximin.

Table 7: Multivariate analysis for bacteremia and serious bacterial infection

Variable	Parameter estimate	Standard error	Odds ratio	95% CI
Bacteremia				
Rifaximin	-1.27	0.88	0.28	0.05 – 1.60
Prophylaxis[1]	-2.16	0.91	0.12	0.02 – 0.68
Alternate donor	0.07	0.72	1.1	0.27 – 4.37
Serious bacterial infections				
Rifaximin	-0.82	0.75	0.44	0.10 – 1.90
Prophylaxis	-1.37	0.79	0.26	0.05 – 1.21
Alternate donor	0.54	0.70	1.71	0.44 – 6.72

[1]Prophylaxis, controls for the use of other prophylactic antibiotics as well as older age (age >18 years) since all adult patients were placed on prophylactic antibiotics.