Bone Marrow Transplantation (2016) **51,** 1087–1092

www.nature.com/bmt

ORIGINAL ARTICLE

Rifaximin preserves intestinal microbiota balance in patients undergoing allogeneic stem cell transplantation

D Weber[1], PJ Oefner[2], K Dettmer[2], A Hiergeist[3], J Koestler[3], A Gessner[3], M Weber[4], F Stämmler[5], J Hahn[1], D Wolff[1], W Herr[1] and E Holler[1]

Intestinal dysbiosis has been associated with acute gastrointestinal GvHD and poor outcome following allogeneic stem cell transplantation (ASCT). To assess the effect of a switch in 2012 from ciprofloxacin/metronidazole to rifaximin for gut decontamination on intestinal microbiota composition and ASCT outcome, we retrospectively analyzed 394 patients receiving ASCT from September 2008 through June 2015. In 131 and 90 patients, respectively, urinary 3-indoxyl sulfate levels and intestinal enterococcal load were measured before conditioning and weekly within the first 28 days after ASCT. The use of rifaximin correlated with lower enterococcal positivity (6.9 vs 21.9%, $P = 0.05$) and higher urinary 3-indoxyl sulfate concentrations (10.5 vs 4.6 µmoL/mmoL crea, $P < 0.001$) after ASCT. Patients on rifaximin showed lower 1-year transplant-related mortality ($P = 0.04$) and higher overall survival ($P = 0.008$). Treatment of infectious complications with systemic antibiotics did not abrogate the beneficial effects of rifaximin on intestinal microbiota composition in the early course of ASCT and outcome. The data underscore the importance of maintaining a diverse population of symbiotic and mutualistic bacteria in the gut on ASCT outcome.

Bone Marrow Transplantation (2016) **51,** 1087–1092; doi:10.1038/bmt.2016.66; published online 21 March 2016

INTRODUCTION

GvHD of the gastrointestinal (GI) tract is one of the most serious complications following allogeneic stem cell transplantation (ASCT).[1] An impact of intestinal microbiota on the pathophysiology of GI GvHD has been assumed since van Bekkum and colleagues observed, in 1974, that mice kept under germ-free conditions did not develop GI GvHD. Consequently, strategies of total or selective suppression of the intestinal microbial flora have been pursued routinely in patients undergoing ASCT.[2] Later, 16S-rRNA gene sequencing of stool specimens provided deeper insights into the composition of intestinal microbiomes[3] of ASCT-recipients and, in particular, of those experiencing acute GI GvHD. A marked early loss of intestinal microbiome diversity was observed during the course of ASCT. This seemed to be influenced by the use of antibiotics as well as acute GvHD itself.[4,5] Consequently, Taur *et al.* demonstrated an association between loss of bacterial diversity at the time of engraftment and transplant-related mortality (TRM) in the first 3 years post transplant. Low-intestinal microbiome diversity correlated with a significantly worse outcome and the emergence of enterococci. Among cases with favorable outcome, on the other hand, *Clostridiales* mostly of the genus *Blautia* dominated, suggesting an imbalance between protective and pathogenic bacteria as a contributing factor to intestinal inflammation.[5,6] We also found urinary 3-indoxyl sulfate (3-IS), a fermental product of commensal colonic bacteria, to be predictive for outcome after ASCT. High levels of 3-IS were correlated with *Clostridiales*, whereas members of the class of Bacilli were associated with low 3-IS levels.[7]

Rifaximin is a rifamycin-derivative with broad-spectrum activity and negligible intestinal resorption.[8,9] Approved for the treatment of traveler's diarrhea, rifaximin is clinically used to treat a variety of GI disorders, for example, hepatic encephalopathy, colonic diverticular disease, irritable bowel syndrome and inflammatory bowel disease.[10] Recently, rifaximin has been shown to maintain commensal microbiota and to exert anti-inflammatory activities.[11] Based on these first positive reports, we switched our antibacterial strategy from ciprofloxacin/metronidazole to rifaximin in 2012.

This change in gut decontamination forms the basis of the present retrospective analysis of the effects of rifaximin and ciprofloxacin/metronidazole on intestinal microbiota composition as indicated by urinary 3-IS levels and enterococcal positivity, as well as on rates of infectious complications and TRM. Our findings reveal a more balanced gut flora and better outcome in patients treated with rifaximin without increasing the risk of infection.

PATIENTS AND METHODS

Patients

A total of 394 adult patients undergoing ASCT between September 2008 and June 2015 at the University Medical Center of Regensburg were retrospectively analyzed, and in a subset of patients a prospective microbiome analysis was performed. Our analyses had been approved by the Ethics Committee of the University Medical Center of Regensburg. After written informed consent, in these patients stool and urinary specimens were collected at a minimum of six different time-points between admission until day 28 after ASCT: before admission, at least once between days −2 to +2, +2 and +10, +11 to +17, +18 to +24 and +25 to +30, respectively. All specimens were stored at −80 °C until analysis.

In our cohort 200 patients received ciprofloxacin 500 mg twice a day and metronidazole 400 mg thrice a day starting typically 8 days before ASCT until 14 days after engraftment. Starting in April 2012, rifaximin

[1]Department of Hematology and Oncology, Internal Medicine III, University Medical Center, Regensburg, Germany; [2]Chair and Institute of Functional Genomics, University of Regensburg, Regensburg, Germany; [3]Institute of Clinical Microbiology and Hygiene, University of Regensburg, Regensburg, Germany; [4]Department of Orthopedic Surgery, University Medical Center, Regensburg, Germany and [5]Chair of Statistical Bioinformatics, Institute of Functional Genomics, University of Regensburg, Regensburg, Germany. Correspondence: Dr D Weber, Department of Hematology and Oncology, Internal Medicine III, University Medical Center Regensburg, Franz-Josef-Strauß-Allee 11, Regensburg 93053, Germany.
E-mail: Daniela.Weber@ukr.de
Received 22 October 2015; revised 24 January 2016; accepted 9 February 2016; published online 21 March 2016

Table 1. Summary of patient characteristics

	Rifaximin (n = *194*)	*Ciprofloxacin/ metronidazole* (n = *200*)	P-value
Median age (range)	53.5 years (+/ − 10.7)	48.9 years (+/ − 12.0)	< 0.001
Diagnosis			
Acute leukemia	111 (57.2%)	108 (54%)	0.84
Lymphatic neoplasia	48 (24.8%)	56 (28%)	
MDS	21 (10.8%)	18 (9%)	
CML	12 (6.2%)	15 (7.5%)	
Aplastic anemia	2 (1.0%)	3 (1.5%)	
Donor			
Sibling	60 (30.9%)	52 (26%)	0.44
Unrelated donor	134 (69.1%)	148 (74%)	
Conditioning			
RIC	178 (91.8%)	163 (81.5%)	0.003
Standard	16 (8.2%)	37 (18.5%)	

Abbreviations: MDS = myelodysplastic syndrome; RIC = reduced intensity conditioning.

Table 2. Comparison of infectious complications between rifaximin and ciprofloxacin/metronidazole

	Fever of unknown origin	*Documented infections*	*Bacteremia/ septicemia*
Ciprofloxacin/ metronidazole	35.0% (70/200)	39.0% (78/200)	28.5% (57/200)
Rifaximin	30.7% (59/192)	41.1% (79/192)	26.0% (50/192)
P-value	0.39	0.68	0.65

Patients treated with rifaximin did not develop more infectious complications during neutropenia compared to patients treated with standard gut decontamination.

Table 3. Comparison of positivity for both, *E. faecium* and *E. faecalis*, as a function of the type of gut decontamination

	Positivity for one enterococcus or negativity for both germs	*Positivity for both enterococci*	P-*value*
Ciprofloxacin/ metronidazole	78.1% (25/32)	21.9% (7/32)	0.05
Rifaximin	93.1% (54/58)	6.9% (4/58)	

Patients treated with rifaximin developed significantly less often positivity for both germs than patients treated with ciprofloxacin/metronidazole.

200 mg twice a day was administered instead ($n = 194$). Patients' characteristics are shown in Table 1. In cases of neutropenic infections, piperacillin/tazobactam was used for empiric first line therapy followed by meropenem and vancomycin as second line therapy. Overall 90.5% ($n = 181$) of patients with ciprofloxacin/metronidazole and 91.7% ($n = 178$) of patients with rifaximin required antibiotic treatment. The clinical standard of using systemic antibiotics for first and second line therapy did not differ between the two decontamination groups.

Analysis of enterococcal positivity by enterococcal PCR

In a subgroup of 90 patients, fecal specimens were collected starting from conditioning until day 28 after ASCT (rifaximin $n = 58$, ciprofloxacin/ metronidazole $n = 32$). *Enterococcus* (*E.*) *faecium* and *E. faecalis* load was monitored by species-specific quantitative real-time PCR-analysis in collected stool specimens as previously described.[5] Amplified 16S-rDNA PCR products of *E. faecium* and *E. faecalis* cloned into a pGEM-T Easy vector (Invitrogen, Carlsbad, CA, USA) served as calibrators. Enterococcal positivity for both germs was used as an indicator of severe microbiome disruption and was defined by either an increase in enterococcal load or by conversion to positivity until day 28.

Analysis of urinary 3-IS

In 131 patients, urinary 3-IS levels were analyzed by reverse-phase liquid chromatography-electrospray ionization-tandem mass spectrometry as previously described (rifaximin $n = 74$, ciprofloxacin/metronidazole $n = 57$).[7]

Bioinformatics and data analysis

Normally and non-normally distributed continuous data are presented as mean (SD) or median (range), respectively. Accordingly, group comparisons were performed by two-sided *t*-test, Mann–Whitney *U*-test or Kruskal–Wallis test. Absolute and relative frequencies were given for categorical data and compared between study groups by Fisher's exact test. All hypotheses were tested in an exploratory manner on a two-sided 5% significance level. Kaplan–Meier analysis was performed to assess outcome. Cox regression was used for multivariate assessment of risk factors. IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) was used for analysis.

RESULTS

Gut decontamination with rifaximin is not associated with higher rates of infectious complications

Regarding neutropenic infections, we observed no differences between rifaximin and ciprofloxacin/metronidazole for fever of unknown origin ($P = 0.39$), documented infections ($P = 0.68$) and bacteremia/septicemia ($P = 0.65$), respectively (Table 2). Among the documented pathogens, the frequencies of Gram-negative (27.4% rifaximin vs 29.4% ciprofloxacin/metronidazole) and Gram-positive (61.6% rifaximin vs 64.7% ciprofloxacin/metronidazole) pathogens were comparable between both groups ($P = 0.56$). Altogether 35.6% of patients on rifaximin compared to 30.9% of patients on ciprofloxacin/metronidazole received only antibiotic first line therapy, whereas 59.2% of rifaximin patients compared to 59.0% of ciprofloxacin/metronidazole patients were treated with additional second or third line regimens ($P = 0.17$).

Rifaximin correlates with markers of high-intestinal microbiome diversity and diminishes the negative effect of systemic antibiotics on microbial composition

Stool specimens from patients receiving rifaximin for gut decontamination, were significantly less often positive for both, *E. faecium* and *E. faecalis* (6.9%, 4/58), in the first 28 days after ASCT than those treated with ciprofloxacin/metronidazole (21.9%, 7/32, $P = 0.05$, Table 3). Although the majority of patients received

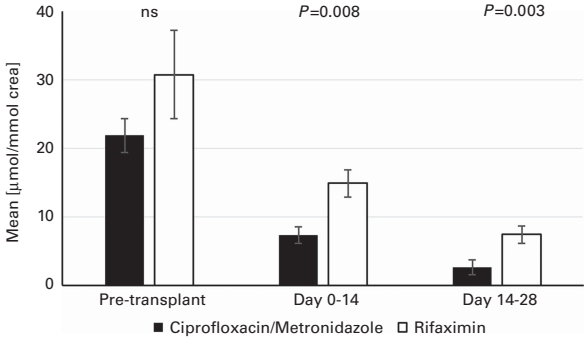

Figure 1. Levels of urinary 3-IS during the course of ASCT in relation to the type of gut decontamination. Patients treated with rifaximin for gut decontamination showed significant higher levels of 3-IS between days 0–14 ($P = 0.008$) and days 14–28 ($P = 0.003$).

during that time also systemic antibiotics to treat infections, we reanalyzed the data excluding those that had not received systemic antibiotics. Still we observed a tendency for patients treated with rifamixin to be less frequently positive for both germs (7.3%, 4/55) than those receiving ciprofloxacin/metronidazole (22.2%, 6/27, $P = 0.07$).

Further, 3-IS levels over the first 4 weeks after ASCT were on average significantly higher in patients receiving rifaximin (10.5 µmoL/mmoL crea, range 0–101.9 µmoL/mmoL crea) than in those receiving ciprofloxacin/metronidazole (4.6 µmoL/mmoL crea, range 0–32.1 µmoL/mmoL crea, $P < 0.001$). This effect was apparent for both days 0–14 ($P = 0.008$) and days 14–28 ($P = 0.003$), whereas before ASCT no significant difference in urinary 3-IS levels was observed between the two groups (Figure 1). This effect was still apparent even when adjusting 3-IS concentrations for preconditioning values. 3-IS levels dropped by 33.7% in the rifaximin vs 73.4% in the ciprofloxacin/metronidazole group ($P = 0.03$) for days 0–14, and by 84.7% for rifaximin vs 99.8% for ciprofloxacin/metronidazole for days 14–28 ($P < 0.001$), respectively. As mentioned before, administration of additional systemic antibiotics did not differ between groups. Although limited by the small number of patients, the highest levels of 3-IS were measured in patients receiving rifaximin without systemic antibiotics, whereas lowest concentrations of 3-IS were detected in those treated with systemic antibiotics in addition to ciprofloxacin/metronidazole. These differences were similar for days 0–14 ($P = 0.005$) and days 14–28 ($P = 0.008$), respectively, after ASCT, whereas before ASCT 3-IS concentrations were comparable. Furthermore, patients on rifaximin taking additional antibiotics still had higher urinary 3-IS levels between days 0–14 ($P = 0.03$) than patients receiving systemic antibiotics in addition to ciprofloxacin/metronidazole. This protective effect of rifaximin was still apparent beyond day 14 after ASCT ($P = 0.002$, Supplementary Figure 1).

TRM and overall survival (OS) appear to be affected by the use of rifaximin

Both a lower TRM (log rank = 0.04) and a higher OS (log rank = 0.008) within the first 12 months were observed in patients taking rifaximin compared to those on ciprofloxacin/metronidazole. Figures 2 and 3 show the respective Kaplan–Meier estimates of TRM and OS for each group. Considering both antibiotic prophylaxis and additional systemic antibiotic treatment, we found a significantly higher TRM in patients treated with both ciprofloxacin/metronidazole and systemic antibiotics compared to patients receiving rifaximin without ($P = 0.03$) and with additional systemic antibiotics ($P = 0.02$), respectively. The probability of transplant-related death was comparable between patients treated with rifaximin and systemic antibiotics and those that received ciprofloxacin/metronidazole only (Figure 4).

We observed severe intestinal GvHD (stage II–IV) rates of 5.3% (1/19) for rifaximin only, 15.5% (25/161) for rifaximin and systemic antibiotic treatment, 10.0% (3/30) for ciprofloxacin/metronidazole without and 22.5% (36/160) with additional systemic antibiotic treatment. Focusing on GI GvHD-related TRM, patients treated with rifaximin succumbed less frequently to GvHD-related TRM (9.3%, 18/194) than patients receiving ciprofloxacin/metronidazole (24.5%, 49/200, $P < 0.001$). The positive effect of rifaximin on both TRM ($P = 0.04$) and OS ($P = 0.02$) persisted until the most recent follow-up in June 2015. Table 4 shows the results of the Cox proportional hazards analysis for TRM. The type of gut decontamination ($P = 0.006$), but also patients' age ($P = 0.001$) and stage of underlying disease ($P = 0.01$) were significantly associated with increased risk of TRM.

DISCUSSION

Since Beelen *et al.*[12] reported in 1999, that antimicrobial chemotherapy targeted at intestinal anaerobic bacteria significantly reduced the severity of acute GVHD in ASCT recipients, gut decontamination with ciprofloxacin/metronidazole has been the standard of clinical care at many transplant centers. Here we report our recent experience with rifaximin for gut decontamination and its impact on intestinal microbiota compared to ciprofloxacin/metronidazole. Patients treated with rifaximin showed higher urinary 3-IS levels and less enterococcal positivity at the time of and after ASCT, irrespective of the additional use of systemic antibiotics to treat infections. In spite of higher mean age in the rifaximin group, patients receiving rifaximin experienced better short- and long-term outcome after ASCT than patients with ciprofloxacin/metronidazole. This is in line with our previous study that found low urinary 3-IS levels early after ASCT prognostic of poor outcome and more transplant-related complications.[7] Despite its effect being restricted to the intestine, as rifaximin is not absorbed, its use did not result in more infectious complications than the administration of ciprofloxacin/metronidazole. Interestingly, the negative impact of prophylactic and systemic antibiotics seemed to be additive, as patients receiving ciprofloxacin/metronidazole and systemic antibiotics had the lowest 3-IS levels and the highest TRM, whereas patients on rifaximin without systemic antibiotics showed the highest 3-IS levels and the lowest risk for TRM.

Our analysis has some limitations: First, it is a sequential cohort analysis, and other factors, such as supportive treatment, may have contributed to the observed effect. Second, in 2012 we also started a supplementation with cholecalciferol (vitamin D), because a potential impact of vitamin D on immunoregulation and maintenance of bacterial diversity has been reported.[13] Nevertheless, our observations suggest possible preservation of pre-transplant intestinal microbiota diversity and, thereby, possibly prevention of inflammatory processes by the use of rifaximin.

Recently, an increasing number of studies have appeared in support of dysbiosis playing an important role in the pathophysiology of inflammatory processes in the GI tract,[14] with the phylogenetic structure of the intestinal microbiota serving as a surrogate of both the severity of inflammation and therapeutic response in patients with inflammatory bowel disease.[15] In the case of ASCT, recent data strongly indicate a correlation between the loss of a balanced gut microbiota with a concomitant shift to potentially pathogenic bacteria, such as enterococci and the risk of developing acute GI GvHD and long-term clinical outcome.[4,5,16]

A gut flora, in contrast, dominated by mutualistic bacteria from the *Clostridium* clusters IV and XIVa seems to exert anti-inflammatory effects by promoting among others regulatory T-lymphocyte responses.[17] Our growing understanding of microbial influences in the pathogenesis of inflammatory diseases

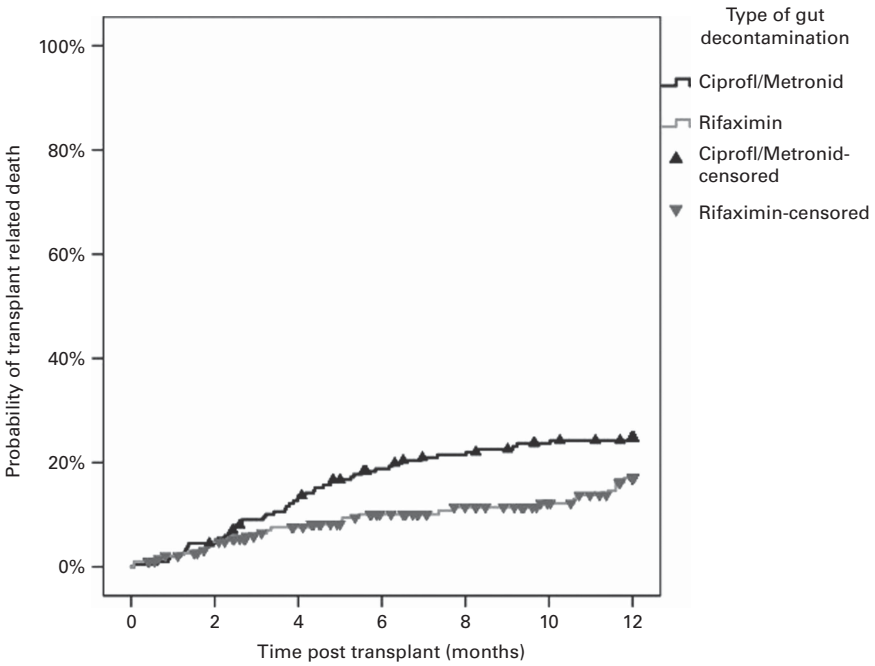

Figure 2. TRM within the first 12 months after ASCT in relation to the type of gut decontamination. Patients treated with rifaximin showed lower TRM than patients on ciprofloxacin/metronidazole ($P = 0.04$).

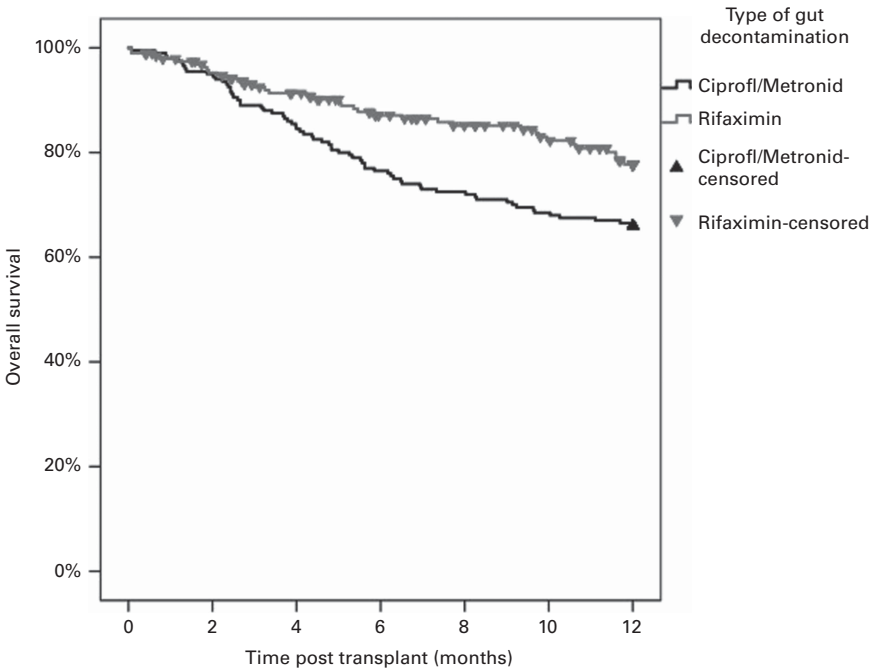

Figure 3. OS within the first 12 months after ASCT in relation to the type of gut decontamination. Patients treated with rifaximin showed a higher OS than patients on ciprofloxacin/metronidazole ($P = 0.008$).

should lead therefore to the development of therapeutic strategies aimed at protecting a balanced intestinal microbiota.

Recently rifaximin, a rifamycin-derivative, has gained interest for the therapy of a variety of GI disorders, including traveler's diarrhea, hepatic encephalopathy, irritable bowel syndrome and inflammatory bowel disease.[18] Several clinical studies have demonstrated the therapeutic efficacy of rifaximin in inducing clinical remission in patients affected by inflammatory bowel disease.[19,20] As Maccaferri *et al.* demonstrated, rifaximin does not affect the overall composition of the human colonic microbiota. It was even shown to increase the fraction of potentially protective bacteria such as various species of the genera *Bifidobacterium* and *Atopobium* of the class *Actinobacteria* and *Faecalibacterium prausnitzii* of the *Clostridium* cluster IV.[11] Further, rifaximin promotes the production of short-chain fatty acids, which are capable of inducing regulatory T cells.[11] In addition, it modulates intestinal inflammation by reducing the expression of bacterial virulence factors, adhesion and epithelial internalization of pathogens and inflammatory cytokine production.[21–23] It has been reported that rifaximin is able to modulate intestinal

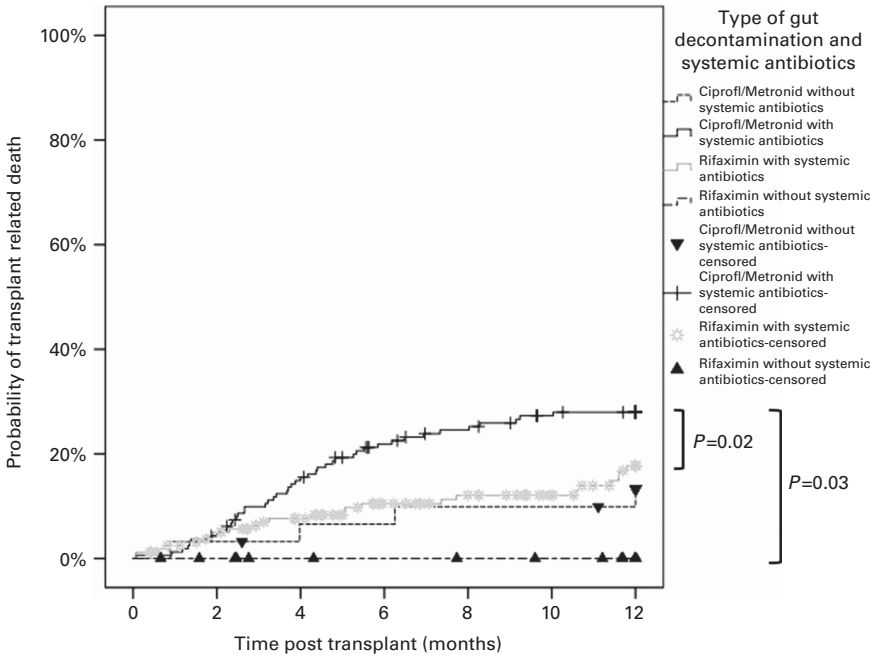

Figure 4. TRM within the first 12 months after ASCT in relation to the type of gut decontamination and systemic antibiotic treatment. Patients treated with rifaximin without systemic antibiotics showed lower TRM than patients treated with ciprofloxacin/metronidazole and systemic antibiotics in addition ($P = 0.03$).

Table 4. Multivariate risk factor analysis for TRM in the first 12 months after ASCT

Risk factor	*P-value*	*HR*	*95% CI for HR*
Type of gut decontamination (rifaximin, $n = 194$)	0.006	0.51	0.31–0.82
Patient's age (age > 50 years, $n = 244$)	0.001	2.87	1.56–5.31
Donor type (MUD, $n = 282$)	0.25	1.31	0.83–2.06
Type of underlying disease (acute leukemia, $n = 219$)	0.07	1.18	0.99–1.41
Stage of underlying disease (advanced, $n = 184$)	0.01	1.90	1.16–3.11
Conditioning (full intensity, $n = 53$)	0.61	1.20	0.56–2.70

Abbreviations: CI = confidence interval; HR = hazard ratio; MUD = matched-unrelated donor. Type of gut decontamination, patients' age as well as stage of underlying disease were significantly associated with increased risk of TRM. In the table, numbers for high-risk groups are indicated for categorical variables. Significance level < 0.05.

microbiota and GI host-cell function by inducing the pregnane-X-receptor, which plays an important role in the induction of genes involved in drug transport and metabolism.[24] The mechanism of the protective effect of pregnane-X-receptor activation is not fully understood, but is in part due to the attenuation of nuclear factor-κB signaling that results in lower expression of pro-inflammatory cytokines.[23–25] This accords with the observation of Quayed *et al.*,[26] who found reduced plasma levels of IL-6 in adolescents treated with rifaximin during ASCT. However, further studies are required to confirm the beneficial effect of rifaximin on outcome after ASCT and to investigate the mechanisms whereby rifaximin modulates microbiota diversity and regulates intestinal inflammation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by the German Research Foundation (DFG, KFO 'Elite'). We acknowledge the help of Heike Bremm, Constanze Winter and Yvonne Schumann in collecting and cryopreserving patient specimens as well as Nadine Nuernberger in performing 3-IS analyses. This work was partially supported by grants from the Regensburg Center for Interventional Immunology and the Marie Curie Initial Training Network Celleurope, European Commission. The project upon which this publication is based was partially supported by grants from the University of Regensburg, Medical Center (ReForM). DW received support from the German Jose Carreras Foundation.

AUTHOR CONTRIBUTIONS

DW, EH and WH were involved in conception and design of the study. DW and JH were responsible for collection of specimens. PO and KD performed measurements of 3-IS levels. AH, JK and AG conducted enterococcal analysis. MW contributed to statistic data analysis. DW and EH collected and analyzed clinical data and wrote the manuscript. All authors read and corrected the final draft.

REFERENCES

1 Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet* 2009; **373**: 1550–1561.
2 van Bekkum DW, Roodenburg J, Heidt PJ, van der Waaij D. Mitigation of secondary disease of allogeneic mouse radiation chimeras by modification of the intestinal microflora. *J Natl Cancer Inst* 1974; **52**: 401–404.
3 Blaser MJ. The microbiome revolution. *J Clin Invest* 2014; **124**: 4162–4165.
4 Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA *et al.* Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp Med* 2012; **209**: 903–911.
5 Holler E, Butzhammer P, Schmid K, Hundsrucker C, Koestler J, Peter K *et al.* Metagenomic analysis of the stool microbiome in patients receiving allogeneic SCT: Loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal GvHD. *Biol Blood Marrow Transplant* 2014; **20**: 640–645.
6 Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L *et al.* The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood* 2014; **124**: 1174–1182.

7 Weber D, Oefner PJ, Hiergeist A, Koestler J, Gessner A, Weber M *et al.* Low urinary indoxyl sulfate levels early after ASCT reflect a disrupted microbiome and are associated with poor outcome. *Blood* 2015; **126**: 1723–1728.
8 Scarpignato C, Pelosini I. Rifaximin, a poorly absorbed antibiotic: pharmacology and clinical potential. *Chemotherapy* 2005; **51**: 36–66.
9 Ojetti V, Lauritano EC, Barbaro F, Migneco A, Ainora ME, Fontana L *et al.* Rifaximin pharmacology and clinical implications. *Expert Opin Drug Metab Toxicol* 2009; **5**: 675–682.
10 Koo HL, DuPont HL. Rifaximin: a unique gastrointestinal-selective antibiotic for enteric diseases. *Curr Opin Gastroenterol* 2010; **26**: 17–25.
11 Maccaferri S, Vitali B, Klinder A, Kolida S, Ndagijimana M, Laghi L *et al.* Rifaximin modulates the colonic microbiota of patients with Crohn's disease: an in vitro approach using a continuous culture colonic model system. *J Antimicrob Chemother* 2010; **65**: 2556–2565.
12 Beelen DW, Elmaagacli A, Muller KD, Hirche H, Schaefer UW. Influence of intestinal bacterial decontamination using metronidazole and ciprofloxacin or ciprofloxacin alone on the development of acute graft-versus-host disease after marrow transplantation in patients with hematologic malignancies: final results and long-term follow-up of an open-label prospective randomized trial. *Blood* 1999; **93**: 3267–3275.
13 Kreutz M, Eissner G, Hahn J, Andreesen R, Drobnik W, Holler E. Variations in 1 alpha,25-dihydroxyvitamin D3 and 25-hydroxyvitamin D3 serum levels during allogeneic bone marrow transplantation. *Bone Marrow transplantation* 2004; **33**: 871–873.
14 Hold GL, Smith M, Grange C, Watt ER, El-Omar EM, Mukhopadhya I. Role of the gut microbiota in inflammatory bowel disease pathogenesis: what have we learnt in the past 10 years? *World J Gastroenterol* 2014; **20**: 1192–1210.
15 Kolho KL, Korpela K, Jaakkola T, Pichai MV, Zoetendal EG, Salonen A *et al.* Fecal microbiota in pediatric inflammatory bowel disease and its relation to inflammation. *Am J Gastroenterol* 2015; **110**: 921–930.
16 Eriguchi Y, Takashima S, Oka H, Shimoji S, Nakamura K, Uryu H *et al.* Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of alpha-defensins. *Blood* 2012; **120**: 223–231.
17 Hansen JJ. Immune responses to intestinal microbes in inflammatory bowel diseases. *Curr Allergy Asthma Rep* 2015; **15**: 61.
18 Gao J, Gillilland MG 3rd, Owyang C. Rifaximin, gut microbes and mucosal inflammation: unraveling a complex relationship. *Gut Microbes* 2014; **5**: 571–575.
19 Prantera C, Lochs H, Campieri M, Scribano ML, Sturniolo GC, Castiglione F *et al.* Antibiotic treatment of Crohn's disease: results of a multicentre, double blind, randomized, placebo-controlled trial with rifaximin. *Aliment Pharmacol Ther* 2006; **23**: 1117–1125.
20 Shafran I, Johnson LK. An open-label evaluation of rifaximin in the treatment of active Crohn's disease. *Curr Med Res Opin* 2005; **21**: 1165–1169.
21 Jiang ZD, Ke S, Dupont HL. Rifaximin-induced alteration of virulence of diarrhoea-producing *Escherichia coli* and *Shigella sonnei*. *Int J Antimicrob Agents* 2010; **35**: 278–281.
22 Brown EL, Xue Q, Jiang ZD, Xu Y, Dupont HL. Pretreatment of epithelial cells with rifaximin alters bacterial attachment and internalization profiles. *Antimicrob Agents Chemother* 2010; **54**: 388–396.
23 DuPont HL. Therapeutic effects and mechanisms of action of rifaximin in gastrointestinal diseases. *Mayo Clin Proc* 2015; **90**: 1116–1124.
24 Hirota SA. Understanding the molecular mechanisms of rifaximin in the treatment of gastrointestinal disorders—a focus on the modulation of host tissue function. *Mini Rev Med Chem* 2015; **16**: 206–217.
25 Cheng J, Shah YM, Gonzalez FJ. Pregnane X receptor as a target for treatment of inflammatory bowel disorders. *Trends Pharmacol Sci* 2012; **33**: 323–330.
26 Qayed M, Langston A, Chiang KY, August K, Hilinski JA, Cole CR *et al.* Rifaximin for preventing acute graft-versus-host disease: impact on plasma markers of inflammation and T-cell activation. *J Pediatr Hematol Oncol* 2013; **35**: e149–e152.

Supplementary Information accompanies this paper on Bone Marrow Transplantation website (http://www.nature.com/bmt)