REGULAR ARTICLE

blood advances

Reg3α concentrations at day of allogeneic stem cell transplantation predict outcome and correlate with early antibiotic use

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Key Points

- Reg3α concentrations on day of graft infusion predict outcome after ASCT.
- Reg3α concentrations are influenced by intestinal dysbiosis induced by early AB use.

Intestinal microbiome diversity plays an important role in the pathophysiology of acute gastrointestinal (GI) graft-versus-host disease (GVHD) and influences the outcome of patients after allogeneic stem cell transplantation (ASCT). We analyzed clinical data and blood samples taken preconditioning and on the day of ASCT from 587 patients from 7 German centers of the Mount Sinai Acute GVHD International Consortium, dividing them into single-center test (n = 371) and multicenter validation (n = 216) cohorts. Regenerating islet–derived 3α (Reg3α) serum concentration of day 0 correlated with clinical data as well as urinary 3-indoxylsulfate (3-IS) and *Clostridiales* group XIVa, indicators of intestinal microbiome diversity. High Reg3α concentration at day 0 of ASCT was associated with higher 1-year transplant-related mortality (TRM) in both cohorts ($P < .001$). Cox regression analysis revealed high Reg3α at day 0 as an independent prognostic factor for 1-year TRM. Multivariable analysis showed an independent correlation of high Reg3α concentrations at day 0 with early systemic antibiotic (AB) treatment. Urinary 3-IS ($P = .04$) and *Clostridiales* group XIVa ($P = .004$) were lower in patients with high vs those with low day 0 Reg3α concentrations. In contrast, Reg3α concentrations before conditioning therapy correlated neither with TRM nor disease or treatment-related parameters. Reg3α, a known biomarker of acute GI GVHD correlates with intestinal dysbiosis, induced by early AB treatment in the period of pretransplant conditioning. Serum concentrations of Reg3α measured on the day of graft infusion are predictive of the risk for TRM of ASCT recipients.

Submitted 5 July 2022; accepted 24 October 2022; prepublished online on *Blood Advances* First Edition 9 November 2022; final version published online 3 April 2023. https://doi.org/10.1182/bloodadvances.2022008480.

Data were analyzed by the authors of this article. All authors confirm access to clinical trial data upon request.

Data are available on request from the corresponding author, Daniela Weber (daniela.weber@klinik.uni-regensburg.de).

The full-text version of this article contains a data supplement.

Introduction

Allogeneic stem cell transplantation (ASCT) is a curative treatment option for a variety of malignant and nonmalignant hematologic conditions, but a significant risk for life-threatening complications remains. Acute graft-versus-host disease (aGVHD) is the major cause of morbidity and mortality. Gastrointestinal (GI) involvement of aGVHD is a major therapeutic challenge, particularly in cases refractory to high-dose corticosteroids, the standard therapy of aGVHD. The mortality of patients with severe steroid-refractory aGVHD of the GI tract is still high (60%-80%), either due to GVHD itself or owing to infectious complications as a result of prolonged immunosuppression.[1-3] Growing evidence shows a crucial role of intestinal microbiota in the pathophysiology of aGVHD.[4,5] Although ASCT is often associated with a loss of intestinal microbiome diversity, this loss is even more pronounced in patients that develop intestinal aGVHD. Changes in microbiota composition mainly consist of a reduction in protective commensal bacteria such as *Clostridiales* and an overgrowth of potentially pathogenic bacteria such as enterococci.[5-7] Intestinal dysbiosis including a reduction in protective metabolites such as short chain fatty acids (SCFAs), indoles, etc, is associated with inflammatory conditions in the GI tract.[8-10] Several single-center studies[4,5,9,10] and 1 large multicenter study have confirmed a relationship between microbiota disruption and poor patient outcome after ASCT independent of geographic and institutional variations.[11] Early and prolonged antibiotic (AB) exposure, conditioning regimen toxicity, as well as an impaired oral intake have been identified as major risk factors of microbiome disturbance.[8] It is therefore of great interest to identify biomarkers of aGVHD that reflect damage to the GI tract as early as possible and before onset of intestinal symptoms. Regenerating islet–derived 3α (Reg3α) is an antimicrobial peptide primarily produced by Paneth cells (PCs), which protects the GI epithelium from gram-positive bacteria. aGVHD damages the intestinal mucosa resulting in an increase in Reg3α concentration in the bloodstream.[12]

Here, we test the hypothesis that intestinal damage indicated by Reg3α serum concentrations[13] can be detected as early as day 0 (day of transplantation) before onset of alloreactivity in a multicenter study including 587 patients undergoing ASCT. Furthermore, we investigated Reg3α, the serum biomarker of acute GI GVHD and indicator of PC damage, measured on the day of transplantation as predictor of transplant-related mortality (TRM).

Methods

Study design

This analysis used the Mount Sinai Acute GVHD International Consortium (MAGIC) database and biorepository for a simultaneous prospective assessment of clinical data as well as biomarkers to develop early, biomarker-based therapeutic strategies. In 2016, 7 German SCT centers constituted MAGIC Germany and followed a rigorous probe study design funded by the German Jose-Carreras Leukemia Foundation (DJCLS 01 GVHD/2016) in order to improve the biomarker-based risk scores[14] through a combination with microbiome parameters. This database of MAGIC Germany comprises data of 587 patients undergoing ASCT in Regensburg (n = 371) and 6 further German transplant centers (Hamburg, n = 119; Erlangen, n = 45; Würzburg; n = 18; Freiburg, n = 8; Frankfurt, n = 9; and Hannover, n = 17;) (supplemental Table 1). Inclusion criteria were hematologic diseases requiring ASCT and receipt of T-cell–repleted grafts. We first analyzed a test cohort of 371 ASCT recipients enrolled between 2008 and 2020 in Regensburg to define a benchmark for Reg3α regarding 1-year TRM. We then validated these findings in a multicenter cohort consisting of 216 patients who underwent transplant between 2012 and 2021 in 6 MAGIC Germany sites (excluding Regensburg). All further analyses were performed using the entire cohort.

With approval by the ethics committee of the MAGIC consortium (number 21-2521-101) and after receipt of written informed consent, blood samples of all 587 patients were collected before start of conditioning therapy (within 3 days before conditioning) and on the day of SCT, which was defined as the time period between days −1 to +1. Urinary and fecal specimens were collected at day +7 range (days +2 to +10). All specimens were stored at −80 °C until analysis.

During the course of ASCT, patients commonly received prophylactic ABs from the start of conditioning until engraftment, but the type of prophylactic AB differed between the centers. In Regensburg, ciprofloxacin 500 mg twice daily and metronidazole 400 mg 3 times daily were administered orally until March 2012 and oral rifaximin 200 mg twice daily thereafter to control the emergence of vancomycin-resistant enterococci. Erlangen also used rifaximin 200 mg twice daily for gut decontamination; patients in Frankfurt, Freiburg, and Würzburg received no AB prophylaxis at all. Hamburg and Hannover used prophylactic ciprofloxacin 500 mg twice daily and levofloxacin 400 mg daily in addition to metronidazole 400 mg 3 times daily, respectively (supplemental Table 1).

Treatment of neutropenic fever/infections with additional systemic broad-spectrum ABs was comparable between the single-center test cohort and the multicenter validation cohort. All patients received first- and second-line treatment of neutropenic fever/infections according to international guidelines[15,16] and the clinical criteria for initiation of first- and second-line ABs were comparable between centers. Piperacillin/tazobactam at a thrice daily dose of 4.0/.5 g was used as empiric first-line therapy, whereas meropenem 1.0 g thrice daily and vancomycin 1.0 g twice daily served as second-line therapy. In cases of penicillin intolerance, patients received alternative first-line treatment with ceftazidime. In the test and validation cohorts, 93.9% and 95.4% of patients, respectively, received additional AB treatment beyond prophylactic regimens. Children received a similar but weight-adapted AB regimen.

To investigate the effect of dysbiosis caused by initiation of systemic broad-spectrum ABs, we classified patients into 2 groups according to the initiation of additional ABs: the early group began AB treatment before the day of ASCT and the late group began AB therapy on or after the day of transplant. In the test cohort, 39.9% of patients received early AB treatment compared with 66.2% in the validation cohort ($P < .001$). However, the incidence of documented bacteremia or sepsis was even less in the early AB group. Further patient characteristics are listed in Table 1. In Regensburg and at all German MAGIC sites patients with unrelated donor grafts routinely received antithymocyte globulin (ATG) as part of GVHD prophylaxis before ASCT in contrast to patients treated with grafts from related donors. Fever represents a common side effect of ATG use and thus often favors the use of ABs before graft infusion.

Table 1. Anthropometric characteristics of the test (n = 371) and validation (n = 216) cohorts

	Test cohort, n = 371, %	Validation cohort, n = 216, %	*P*	Total, n = 587, %
Patient age, y				
>50	68.2	62.5	.16	66.1
Patient sex				
Female	35.8	36.6	.86	36.1
Type of underlying disease				
Acute leukemia	51.8	48.1	.40	50.4
Disease stage				
Late	37.5	34.3	.46	36.4
Conditioning			.09	
RIC	85.3	79.6		83.2
Standard	14.4	19.0		16.1
Other	0.3	1.4		0.7
GVHD prophylaxis			<.001	
CNI/MTX	64.0	20.4		47.8
CNI/MMF	18.7	73.6		39.1
Other	17.3	6.0		13.1
Karnofsky Index				
≥90%	61.8	71.90	.03	64.9
Donor				
Family (no ATG)	30.0	25.0	.42	28.2
Gut decontamination			<.001	
Rifaximin/no ABs	87.1	37.7		68.7
Cipro/cipro + metro	12.9	62.3		32.3
Early systemic ABs				
Before day of ASCT	39.9%	66.2%	<.001	49.7%

CNI, calcineurin inhibitors; cipro, ciprofloxacin; metro, metronidazole; MMF, mycophenolate mofetil; MTX, methotrexate; RIC, reduced intensity conditioning.

The primary cause of death was assigned according to a published hierarchy. For cause of death analysis, the previously published hierarchy of death classification according to the MAGIC consortium was used.[17]

Reg3α serum concentrations were analyzed by enzyme-linked immunosorbent assay, as previously described and are reported in nanograms per milliliter (ng/mL).[12]

Urinary 3-indoxylsulfate (3-IS) and creatinine concentrations were determined by reverse-phase liquid chromatography electrospray-ionization tandem mass spectrometry, as previously described.[5]

16S ribosomal RNA (rRNA) gene copy numbers of *Clostridium* cluster XIVa species were determined in fecal DNA preparations by real-time quantitative polymerase chain reaction, as previously described.[10]

Bioinformatics and data analysis

Continuous data are presented as mean (± standard deviation). Group comparisons were performed by Mann-Whitney *U* tests owing to nonnormal data distribution. Absolute and relative frequencies for categorical data are compared between study groups by χ^2 or Fisher exact tests. All hypotheses were tested with a two-sided 5% significance level. Receiver operating characteristic curve plots were generated and the area under the curve was assessed in both cohorts to evaluate the prediction of 1-year TRM by Reg3α serum concentrations on the day of graft infusion. The Youden Index was calculated to define a Reg3α threshold value. Factors associated with high Reg3α serum concentrations were assessed using logistic regression analysis. Kaplan-Meier analysis was performed to assess TRM, and Cox regression was used for multivariable assessment of risk factors. Competing-risk analysis[18] for Reg3α-related TRM, infectious TRM, and relapse was performed using software package R version 3.2.2 (The R Foundation of Statistical Computing, Vienna, Austria). IBM SPSS Statistics 25 (SPSS Inc, Chicago, IL) was used for all other analyses.

Results

Reg3α serum concentrations at day 0 are associated with TRM

First, we analyzed the ability of Reg3α serum concentrations at day of transplantation to predict 1-year TRM by creating a receiver operating characteristic curve in the test cohort. The area under the curve was 0.64 (95% confidence interval [CI], 0.56-0.72; $P = .001$). A threshold of 110 ng/mL was found to have the highest Youden Index in terms of sensitivity and specificity. Using this threshold, Kaplan-Meier survival plots showed a significant difference between patients with high vs those with low Reg3α values in both the test (33.3% vs 11.8%, $P < .001$) and validation cohorts (21.3% vs 8.4%, $P = .009$) (Figure 1).

Cox regression analysis in the combined test and validation cohorts identified Reg3α at the time of ASCT as an independent prognostic factor for 1-year TRM (hazard ratio, 2.9; 95% CI, 1.8-4.8; $P < .001$) as well as patient age (hazard ratio, 4.0; 95% CI, 1.7-9.4; $P = .002$). Other key parameters such as sex, type/stage of disease, donor type, and Karnofsky Index were not predictive (Table 2). Competing-risk analysis confirmed the association of high Reg3α concentrations at day 0 and TRM when relapse was considered as competing risk ($P < .001$) (Figure 2). TRM was higher in the high-Reg3α group owing to GI GVHD (11.0% vs 5.3%, $P = .005$), infectious complications (8.8% vs 2.4%, $P < .001$), as well as other reasons (8.1% vs 2.7%, $P = .001$) (Table 3; supplemental Figure 1). In contrast, preconditioning Reg3α concentrations showed no correlation to 1-year TRM (log rank = 0.22) (supplemental Figure 2). This was also confirmed in Cox regression analysis (supplemental Table 2).

Early AB treatment independently correlates with day-0 Reg3α concentrations

Intravenous application of broad-spectrum ABs before the day of ASCT correlated with higher Reg3α concentrations at day 0 (112.1 ± 132.4 ng/mL) compared with late or non-AB treatment (63.7 ± 53.3 ng/mL, $P < .001$), whereas no difference was found for pretransplant Reg3α concentrations (42.9 ± 45.1 ng/mL vs 41.6 ± 52.0 ng/mL; nonsignificant) (Figure 3). Similarly, patients that had received ciprofloxacin or combined ciprofloxacin/metronidazole had higher Reg3α serum concentrations (106.4 ± 140.3 ng/mL) than patients receiving rifaximin or no prophylactic gut

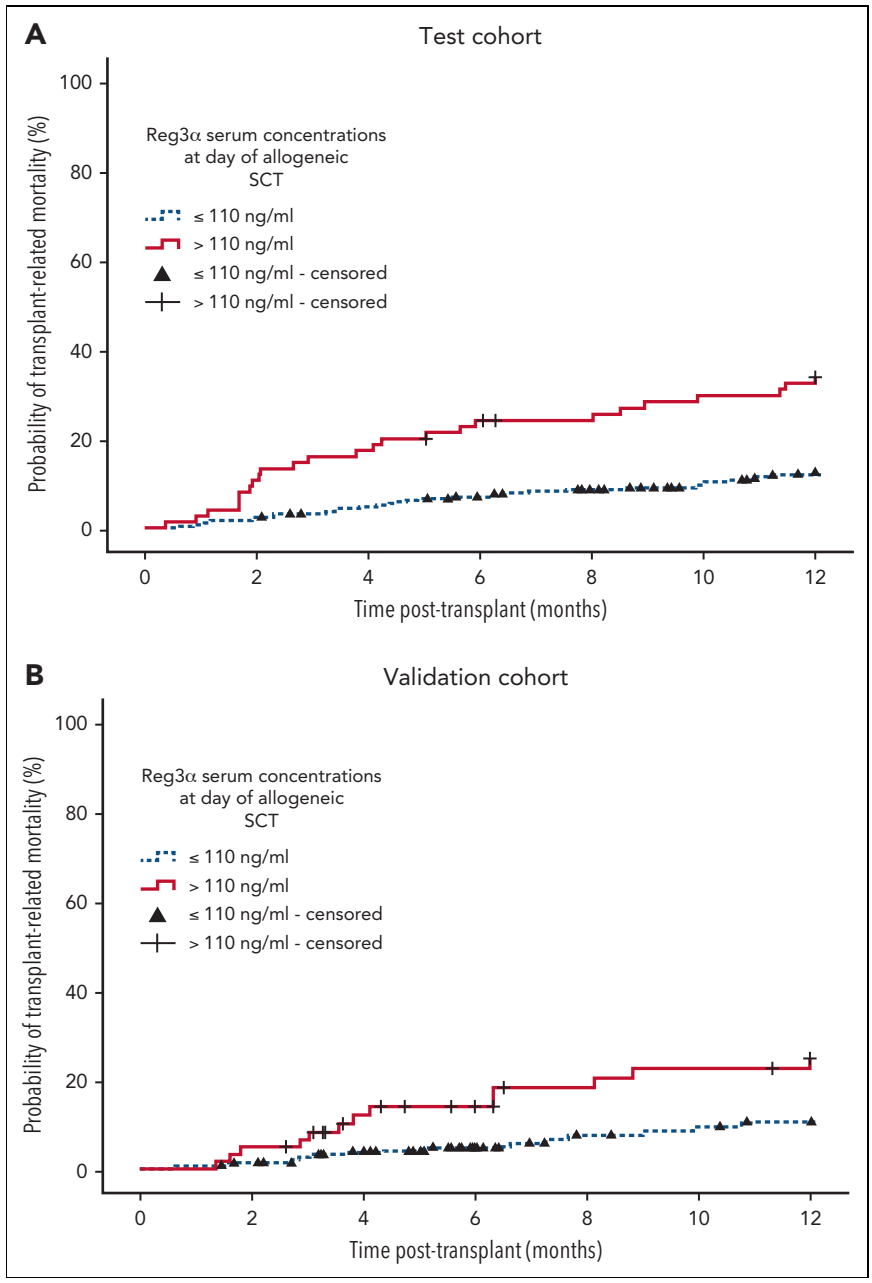

Figure 1. Kaplan-Meier survival plots for 1-year TRM in relation to Reg3α serum concentrations at the time of ASCT. Kaplan-Meier survival plots show a significant difference between patients with high and those with low Reg3α serum values according to a threshold of 110 ng/mL in both the test (n = 371, $P < .001$) (A) and validation cohorts (n = 216, $P = .009$) (B).

decontamination (79.1 ± 80.6 ng/mL, $P = .01$). However, the effect of early systemic broad-spectrum ABs was independent of type of gut decontamination. Reg3α concentrations at day of transplantation were also higher in patients aged >50 years (95.3 ± 115.6 ng/mL) than in younger patients (72.8 ± 73.4 ng/mL, $P = .002$). Higher Reg3α serum concentrations were measured at day of transplantation in patients with unrelated donors (99.3 ± 113.6 ng/mL) compared with related donors (59.3 ± 69.3 ng/mL, $P < .001$). This seemingly contradictory result points toward the differential use of ATG treatment, leading to an earlier use of systemic ABs owing to febrile episodes. Of the patients with unrelated donors, 54% were treated with ABs and had signs of cytokine release syndrome after ATG, whereas only 39% of patients with related donors were treated systemically ($P = .001$). In addition, Reg3α concentrations (94.4 ± 92.7 ng/mL) were higher in patients with a Karnofsky Index of <90% than in patients with a Karnofsky Index of ≥90% (73.9 ± 76.6 ng/mL, $P = .008$). Multivariable analysis confirmed the independent correlation of high Reg3α serum concentrations at day 0 and early systemic ABs (odds ratio, 3.1; 95% CI, 2.0-4.8; $P < .001$), whereas type of gut decontamination, patient age, donor type, and Karnofsky Index revealed no independent correlation (Table 4).

Microbiota disruption by early AB treatment correlates with Reg3α release

Prolonged suppression of commensals by systemic AB treatment before day −3 in relation to ASCT resulted in even higher Reg 3α

Table 2. Cox regression analysis for 1-year TRM in the study cohort

	P	Hazard ratio	95% CI for hazard ratio
Reg3α serum concentrations, ng/mL			
≤110 vs >110	<.001	3.2	1.9-5.2
Patient age, y			
≤50 vs >50	.003	3.8	1.6-9.2
Patient sex			
Female vs male	.8	1.1	0.6-1.8
Type of underlying disease			
Acute leukemia vs no acute leukemia	.7	0.9	0.5-1.5
Stage of disease			
Early vs late	.2	1.4	0.9-2.4
Donor type			
Related (no ATG) vs nonrelated (ATG)	.6	1.1	0.8-1.6
Karnofsky Index, %			
<90 vs ≥90	.4	0.8	0.5-1.4
Conditioning regimen			
RIC vs standard	.7	0.9	0.4-1.8

Parameters included in the model: Reg3α serum concentration, patient's sex, patient's age, type of underlying disease, stage of disease, donor type, Karnofsky Index, and conditioning regimen.

concentrations than in AB treatment after day −3 (114.4 ± 115.8 ng/mL vs 75.4 ± 95.1 ng/mL, $P < .001$) (Figure 4). The administration of carbapenems, known to be the strongest suppressors of commensals, was associated with high Reg3α concentrations (139.2 ± 143.4 vs 70.7 ± 62.1 ng/mL, $P < .001$) compared with all other types of AB treatment.

Urinary 3-IS concentrations on day +7, as a marker for microbiome diversity, were reduced (5.7 ± 18.9 ng/mL) in patients with high

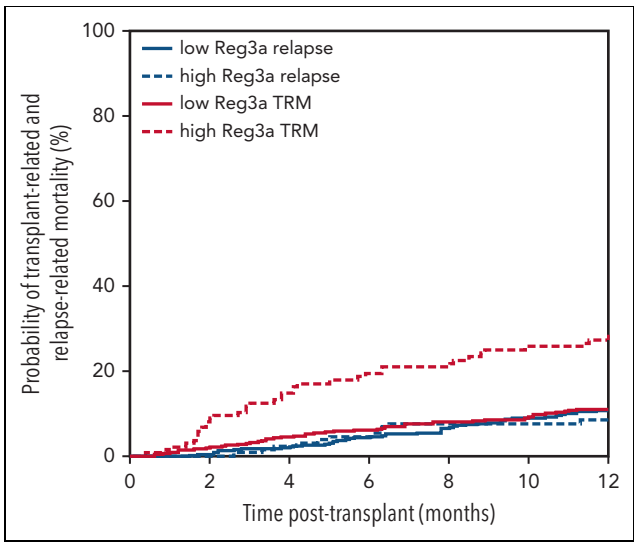

Figure 2. Competing-risk analysis for 1-year TRM and relapse in relation to high Reg3α concentrations. Competing-risk analysis confirmed the association of high Reg3α concentrations at day 0 and TRM when relapse was considered as competing risk.

Reg3α serum concentrations on day 0, whereas patients with low Reg3α concentrations on day 0 had higher urinary 3-IS concentrations (21.7 ± 20.4 ng/mL, $P = .04$; correlation coefficient, −0.21, $P = .001$). Similarly, stool copy numbers of the commensal *Clostridiales* group XIVa revealed lower values in patients with high Reg3α concentrations ($4.9 \times 10^8 \pm 1.5 \times 10^9$ copy numbers) than in patients with low Reg3α concentrations ($1.4 \times 10^9 \pm 2.7 \times 10^9$ copy numbers, $P = .004$; correlation coefficient, −0.15, $P = .04$).

Older patient age is the only independent variable correlating with high Reg3α concentrations before conditioning. Analyzing parameters associated with high Reg3α serum concentrations in the preconditioning situation we identified high patient age ($P = .002$) and low Karnofsky Index ($P = .005$) in univariate analysis. However, only patient age remained as independent parameter correlating with Reg3α serum concentrations in multivariable analysis (Table 5).

Discussion

Here, we report, to the best of our knowledge, for the first time, that early Reg3α serum concentrations measured on the day of transplant predict long-term outcomes of recipients of ASCT: increased concentrations of Reg3α are associated with increased TRM as well as poor overall survival at 1 year. Notably, not only GVHD-induced deaths but also deaths due to infections and organ toxicities were increased in the high-Reg3α group. This could be explained by indirect effects of intensified immunosuppression in these patients or by direct effects of microbiota damage because anti-infectious defense and bacterial translocation as well as epithelial integrity are also directly influenced by the microbiome.[19] Multivariable analysis that included established clinical risk factors showed early AB treatment to be the strongest predictor of early Reg3α release. We observed that high Reg3α concentrations correlated with low concentrations of urinary 3-IS, a metabolic marker indicating an absence of commensal bacteria, as well as decreased stool copy numbers of *Clostridiales*;[5] therefore, these data indicate an association between intestinal dysbiosis and elevated Reg3α concentrations at day 0. This correlation is unlikely related to a preexisting microbiome disturbance because we found no difference in microbiome diversity before conditioning.

Early intestinal dysbiosis and pretransplant conditioning toxicity might damage intestinal mucosa and reduce intestinal epithelial integrity. In addition, pretransplant conditioning may facilitate epithelial injury by dysbiosis. Furthermore, the impaired repair capacity of epithelial cells and intestinal stem cells as a consequence of PC damage may sensitize the epithelial cells for GVHD-induced damage. Increased bacterial translocation by intestinal dysbiosis and epithelial injury may further enhance immunostimulation and GVHD development. Recently, Reddy et al[20] reported that a balanced interaction between immune and tissue tolerance can modulate the immunopathology of aGVHD. The capacity of the parenchymal tissue to maintain intestinal homeostasis and to contribute to tissue resilience and regeneration is an important factor to mitigate GVHD severity and to influence disease outcome.[20]

A balanced microbiome with high percentage of commensal bacteria like *Clostridiales* and their protective metabolites such as

Table 3. Overview of survival and causes of death according to MAGIC hierarchy in relation to Reg3α serum concentrations at day of ASCT

	Low Reg3α, %	High Reg3α, %	Total, %
Survival	79.4	64.0	75.8
Relapse	10.2	8.1	9.7
GVHD*	5.3	11.0	6.6
Infection	2.4	8.8	3.9
Others†	2.7	8.1	3.9

*Organ-specific rates of GVHD: GI GVHD, 87.2%; liver GVHD, 19.0%; and skin GVHD, 71.4%.

†Other: hemorrhage, 22.2%; cardiovascular disease, 14.8%; secondary malignancy, 14.8%; leukoencephalopathy, 22.2%; posttransplant lymphoproliferative disease, 7.4%; and liver toxicity, 11.1%.

SCFA is of great importance for intestinal homeostasis and epithelial integrity.[21,22] In several single-center studies, the detrimental impact of microbiome disruptions on outcome of ASCT recipients was demonstrated.[4,5,10,23] Recently, even a large-scaled multicenter study showed patterns of microbiota disruption and, particularly, a dominance of enterococci to be associated with poor clinical outcomes independent of institutional practices and geographic locations.[11] Several factors, however, can affect this sensitive equilibrium; for example, the use of systemic broad-spectrum ABs has a profound and long-lasting effect on the composition and functionality of human microbiota resulting in higher susceptibility to inflammatory conditions.[24] In ASCT, early beginning of AB treatment before day 0 significantly affects clinical outcomes and GVHD-associated TRM.[9,10,24] As fever is a common side effect of ATG use, when applied before unrelated donor transplantations, it frequently triggers immediate AB treatment, which facilitates early microbiome disruptions and thus increases the risk for transplant-related morbidity and mortality. In this context and on the basis of careful clinical monitoring of septicemia, a more restrictive use of broad-spectrum ABs during ATG therapy could be considered; for example, by delaying start of ABs in the first 12 hours after ATG-related fever, which is frequently triggered by cytokine release.

To date, Reg3α release was considered to be a consequence of GVHD-induced PC damage, in which GVHD was enhanced by early dysbiosis and loss of protective metabolites. Our data, however, suggest that Reg3α release can occur as a direct consequence of early dysbiosis in the period of pretransplant conditioning independently from T-cell activation. PCs are described to play an important role in the effector phase of GI GVHD; destruction of PCs due to cytotoxic T-cell damage was correlated with high disease severity and poor treatment response.[25-27] PCs are crucial in maintaining intestinal homeostasis and promote the regeneration of the intestinal epithelium as well as the modulation of microbial flora by production of antimicrobial peptides like α-defensins and Reg3α.[28-33] In line with PC damage, Reg3α was described as a biomarker at onset of GVHD and systemic Reg3α release explained by microscopic breaches in the mucosal epithelial barrier permitting Reg3α to traverse into the systemic circulation.[12,26] According to our observation, early Reg3α measurements already on the day of transplant may be used as a valuable clinical parameter predicting the outcome of ASCT recipients. Whereas detailed microbiome analyses like 16S rRNA sequencing are expensive, time consuming, and thus usually not practicable in clinical routine, the analysis of Reg3α is well established and provides real-time results. Although previous findings indicate an indirect effect of dysbiosis on PCs via loss of immunoregulation and a subsequently increased crypt and PC destruction by alloreactive T cells, our data suggest that intestinal mucosal damage induced by early dysbiosis after AB treatment during conditioning toxicity might directly cause PC damage and contribute to epithelial barrier disruption that favors transmission of Reg3α into the blood. Because Reg3α concentrations before conditioning therapy neither correlated with TRM nor underlying

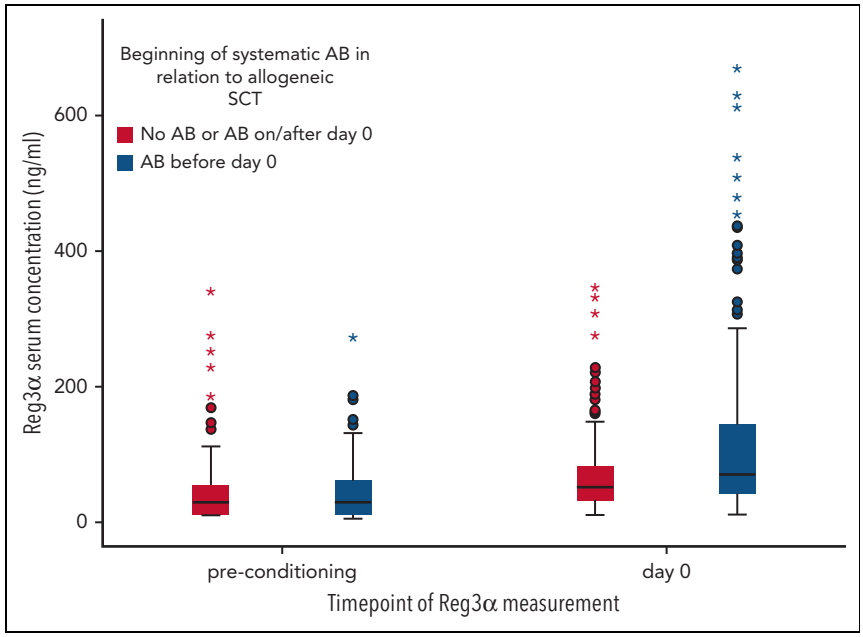

Figure 3. Correlation of Reg3α increase in relation to early AB treatment. The increase in Reg3α concentrations from the pretransplant situation until day 0 was higher in the early ABs group than in patients who received late or no AB treatment ($P = .04$).

Table 4. Logistic regression analysis for parameters correlating with Reg3α serum concentrations at day of ASCT (significant parameters of univariate testing)

Risk factor	*P*	Odds ratio	95% CI
Timing of AB treatment			
Early (before day of ASCT) vs late (on or after day of ASCT)	<.001	3.1	2.0-4.8
Gut decontamination			
Rifaximin/no ABs vs cipro/cipro + metro	.6	1.1	0.7-2.0
Patient age, y			
≤50 vs >50	.3	1.0	1.0-1.0
Donor type			
Related (no ATG) vs unrelated (ATG)	.4	1.2	0.8-1.6
Karnofsky Index, %			
<90 vs ≥90	.3	1.1	0.9-1.5

Parameters included in the model: timing of AB treatment, gut decontamination, patient age, donor type, and Karnofsky Index.

disease or treatment-related parameters, we assume that day0 Reg3α release does not simply indicate higher vulnerability of heavily pretreated patients but is induced by AB treatment in the presence of conditioning toxicity. Several mechanisms could contribute to this early PC damage: first, there is increasing evidence that conditioning by cytotoxic treatment and irradiation results in PC apoptosis;[34-36] and second, dysbiosis itself induced by early use of ABs has been reported to substantially reduce PCs.[37] PC function and survival are regulated, in part, by interleukin 22 (IL-22) signaling, which requires intact commensal bacteria and their protective metabolites such as SCFA. Along these lines, Yang et al demonstrated that SCFA produced by commensal bacteria like *Clostridiales* can promote IL-22 production by innate lymphoid cells and T cells through G protein–coupled receptor 41.[38] In addition, Ghimire et al showed significant suppression of intestinal IL-22 by systemic broad-spectrum ABs in patients suffering from acute GI GVHD.[39] Low numbers of IL-22–secreting innate lymphoid cells as a consequence of AB treatment as well as intestinal dysbiosis with loss of microbiota-derived SCFA can lead to loss of epithelial integrity and again favor Reg3α leakage.[38-40]

Because it is not possible to obtain GI biopsies in this early period of SCT, murine GVHD experiments may help to dissect the mechanisms of early Reg3α release in the future. Interestingly, recipient age emerged as the only additional risk factor for Reg3α increase on day 0 and before conditioning. It is known from literature that age-related changes of intestinal microbiota can promote intestinal inflammation through the production of proinflammatory cytokines,[41] and age-related changes in metabolic signaling can drive the loss of epithelial integrity and homeostasis.[42] In addition, in murine models, adverse effects of aging reduce the expression of key PC-related genes as well as their function and thus negatively affect the regenerative ability of the gut epithelium.[43]

This study has several limitations, the validation cohort shows a variable recruitment of patient numbers between centers and patient heterogeneity. Furthermore, this study is limited to available parameters obtained in the MAGIC Germany consortium. Detailed microbiome analyses using 16S rRNA sequencing are missing. In addition, the current study is only able to show correlations between the different variables and thus does not allow conclusions regarding causality. A strength of this study is the multicenter approach including different types of AB prophylaxis applied in the different MAGIC sites. Although further prospective trials are required to confirm our results, the current data open a new perspective on the role of Reg3α in ASCT. High Reg3α serum concentrations seem to be not only a biomarker for acute GI GVHD but, even more so, an early indicator of intestinal mucosal damage exacerbated by pretransplant conditioning toxicity and intestinal microbiome disruption. Because Reg3α serum concentration on day 0 was highly predictive for 1-year TRM, Reg3α might depict a valuable and clinically useful parameter for early identification of patients at risk of poor outcome after ASCT. An early disruption of the intestinal epithelial barrier function might induce a tissue micromilieu that suppresses tolerance mechanisms, even before the infusion of the donor graft, and thus might increase the susceptibility for GVHD development. However, our observations need to be further elucidated by experimental models focusing on pathophysiologic pathways.

As we have shown previously, intestinal dysbiosis early in the course of an ASCT between day 0 and 10 is crucial to the outcome of ASCT recipients because this time frame represents a vulnerable phase of

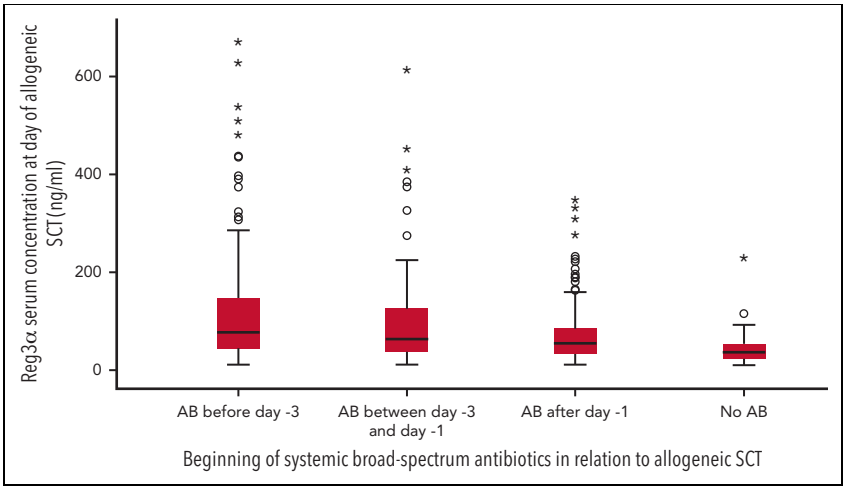

Figure 4. Correlation of Reg3α serum concentration on day 0 in relation to beginning of AB treatment. Prolonged suppression of commensals by systemic AB treatment before day −3 in relation to ASCT resulted in higher Reg 3α concentrations than with AB treatment after day −3 ($P < .001$).

Table 5. Logistic regression analysis for parameters correlating with Reg3α serum concentrations before ASCT

	Univariable		Multivariable	
Risk factor	***P* univariate**	***P***	**Odds ratio**	**95% CI**
Patient age, y				
≤50 vs >50	.00	.05	1.8	1.0-3.4
Patient sex				
Female vs male	.85	.96	1.0	0.6-1.8
Type of underlying disease				
Acute leukemia vs no acute leukemia	.21	.55	1.2	0.7-2.1
Stage of disease				
Early vs late	.47	.67	1.1	0.6-2.0
Donor type				
Related (no ATG) vs unrelated (ATG)	.36	.52	1.2	0.7-1.8
Type of conditioning				
RIC vs standard/other	.1	.76	0.9	0.5-1.7
Type of GVHD prophylaxis				
CNI/MTX vs CNI/MMF/other	.89	.50	1.2	0.8-1.8
Karnofsky Index, %				
<90 vs ≥90	.005	.35	0.8	0.4-1.4
Beginning of AB treatment				
Early (before day of ASCT) vs late (on or after day of ASCT)	.82	.47	1.2	0.7-2.1
Gut decontamination				
Rifaximin/no ABs vs cipro/cipro + metro	.39	.71	1.1	0.7-1.9

Parameters included in the model: patient age, patient sex, type of underlying disease, stage of disease, donor type, type of conditioning, type of GVHD prophylaxis, Karnofsky Index, beginning of AB treatment, and gut decontamination.

immune reconstitution in which changes in the microbiota could trigger long-term complications.[5,10] Our data assume that the stage for a safe ASCT without TRM/GVHD may be set early or even before the infusion of allogeneic cells and might be positively influenced by a balanced microbiome composition; for example, by the very deliberate restrictive use of systemic AB treatment, risk adapted prophylaxis, or even by interventions restoring microbial integrity such as fecal microbiota transplantation.

Acknowledgments

The authors thank Heike Bremm, Yvonne Schuhmann, and Tatjana Schifferstein for collecting and cryopreserving patient specimens and Heike Bremm and George Morales for performing Reg3α analyses. Furthermore, the authors thank all colleagues at German Mount Sinai Acute GVHD International Consortium sites for collecting patient specimens and clinical data.

The German MAGIC consortium was supported by the German Jose-Carreras Leukemia Foundation (DJCLS 01 GVHD/2016) and the German Research Foundation (DFG) via SFB/TRR 221 (project B13). The Reg3α analyses performed at Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai were also supported by a grant from the National Institutes of Health (P01 CA 039542).

Authorship

Contribution: D. Weber, E.H., J.E.L., J.L.M.F., W.H., and M. Edinger were involved in conception and design of the study; D. Wolff, M. Edinger, and H.P. were responsible for collection of specimens; E.H. and J.L.M.F. were responsible for Reg3α measurements; A.H. and A.G. performed bacterial analysis; S. Gleich and R.Y. were responsible for data entry in the MAGIC database; M. Weber contributed to statistical data analysis; F.A., W.R., M. Wölfl, S.K., R.Z., H.B., P.B., E.U., and M. Eder were responsible for collection of patient specimens and clinical data at German MAGIC sites; D. Weber, E.H., E.M., and S. Ghimire collected and analyzed clinical data; D. Weber, M. Weber, J.L.M.F., and E.H. wrote the manuscript; and all authors read and corrected the final draft.

Conflict-of-interest disclosure: E.H. served on the advisory board for Maat Pharma and Pharmabiom. R.Z. declares honoraria from Novartis, Incyte, Sanofi, and Mallinckrodt. D. Wolff declares honoraria from Novartis, Gilead, Takeda, Incyte, Sanofi, and Mallinckrodt. J.E.L. received royalties and is a coinventor on a graft-versus-host disease biomarkers patent; received research support from Biogen, Equillium, Incyte, MaaT Pharma, and Mesoblast; and received consulting fees from Bluebird Bio, Equillium, Jazz, Mallinckrodt/Therakos, Mesoblast, and X4 Pharmaceuticals. J.L.M.F. received royalties and is a coinventor on a graft-versus-host disease biomarkers patent. P.B. declares research grants from Neovii, Riemser, and Medac; serves on the advisory board for Novartis, Cellgene, Amgen, Medac, and Servier; declares honoraria from Miltenyi, Jazz, Riemser, Novartis, as well as Amgen; and received patent and royalties from Medac. None of these stated conflicts of interests are related to this study. The remaining authors declare no competing financial interests.

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