

# Blood and Marrow TRANSPLANTATION

## REVIEWS

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## Symposium Report 2017 BMT TANDEM MEETINGS

### Breakthrough Therapies for Acute Graft-Versus-Host-Disease

Adapted from a continuing medical education plenary session presented at the 2017 BMT Tandem Meetings on February 24, 2017, in Orlando, Florida.

This program is supported by an educational grant from Incyte.

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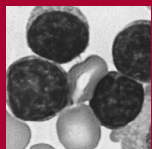
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### Program Overview

This activity will provide focused instruction to healthcare professionals caring for patients with Graft-Versus-Host Disease (GVHD). The activity is designed to provide clinically relevant

updates on the treatment of patients with hematologic malignancies likely to undergo allogeneic stem cell transplantation. The activity will focus on increasing clinician knowledge and the ability to integrate changes into practice to improve patient outcomes. The program will highlight the changes in treatment that acute GVHD is experiencing due to recent implications of clinical trials and breakthrough designations of target agents. Topics for presentation

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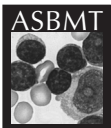
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continued from page 1

will include: the antigens and microbiome characteristics involved in GVHD in addition to biomarkers for GVHD.

### Learning Objectives

Upon successful completion of this educational activity, participants should be better able to:

- Describe the overall clinical understanding of the pathophysiology, guidelines, and patient risk factors for GVHD
- Evaluate novel treatment options, especially breakthrough therapies, for GVHD
- Identify the importance of biomarkers for GVHD and the progress on finding more effective options

### Target Audience

This activity has been developed and is intended for transplant specialists, oncologists, hematologists and other healthcare professionals involved in the treatment of patients with GVHD.

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Motoko Koyama, MD, PhD has nothing to disclose

Ernst Holler, MD, PhD discloses that he has been on the Advisory Board for Pharmacyclics. He also received honoraria from Prime Oncology and Sanofi.

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**FROM THE EDITOR'S DESK**
**Acute Graft-Versus-Host Disease 2.0: Rebooting Approaches to Management Prompted By New Insights into Pathogenesis**

Nosha Farhadfar, MD, John R. Wingard, MD  
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Despite considerable advances in HLA-typing and post-transplant immunosuppressive therapies, acute graft-versus-host disease (GVHD) remains one of the major causes of morbidity and early non-relapse mortality in allogeneic hematopoietic stem-cell transplantation (HSCT) recipients. It has been argued that unless substantial progress is made in prevention or control of GVHD, the ultimate utility of HSCT will be limited. Unfortunately, there has been limited progress in the effective control of GVHD over the past several decades and the major therapies are fraught with suboptimal GVHD control and substantial serious toxicities.

Over the past few decades, high dose systemic corticosteroids has been the mainstay of treatment of acute GVHD. Unfortunately, fewer than half of the patients achieve a durable response to

initial corticosteroid therapy and there are substantial toxicities that compound the risks posed by GVHD. Due to inadequate response to primary therapy, many patients will go on to have additional treatments. Few prospective comparative studies have been done and what trials have been conducted have demonstrated only modest incremental benefits of additional immune-suppressive agents to corticosteroids, including pharmacologic agents, polyclonal or monoclonal antibodies, and extracorporeal photopheresis in this setting. Moreover, survival benefits have not been seen with any second-line treatment. Hence, there is no consensus regarding the "standard of care" second-line therapy for steroid refractory acute GVHD. Patients who fail initial therapy with high dose corticosteroids have dismal prognosis with 1-year mortality rate of 70% to 90%.

Traditionally used diagnostic and staging criteria have lacked the ability to identify those at higher risk for treatment failure and death. Therefore, new approaches to acute GVHD prevention, better tools for risk stratification of patients with acute GVHD and individualized treatment options are urgently needed to improve outcomes after HSCT.

In recent years, improved understanding of the pathophysiology of GVHD, identification of biomarkers to better prognosticate and guide therapies, interesting observations about the link between the gut microbiota and GVHD have provided

new hope for novel approaches to prevent and treat GVHD. These insights have allowed the testing of new agents with novel mechanisms (some developed for other indications, now being repurposed for GVHD). Facilitation of such trials have been aided by the creation of a clinical trials network (the NHLBI/NCI funded Blood and Marrow Transplant Clinical Trials Network) to rapidly conduct rigorous controlled trials to test treatment and prevention strategies as well as to validate biomarkers to create prognostication models that aid individualized treatment approaches. All of these are finally making some promising inroads.

This issue of *Bone and Marrow Transplantation Reviews* contains the transcript of a symposium that took place at the BMT Tandem meeting in February 2017 in Orlando FL, which discussed new insights into the underlying pathophysiology of acute GVHD, the identification of biomarkers of GVHD, the link between changes in the gut microbiota and GVHD, the interaction between alloantigen presentation and GVHD. These are fueling the identification of new therapeutic targets and the development of new predictive models using recently identified biomarkers for risk-stratification of patients which is necessary for development of individualized approaches that hopefully will archive more effective and less toxic therapeutic approaches in this setting.



James Ferrara, MD, DSc

**Introduction**

Graft-versus-host disease (GVHD) is the leading cause of non-relapse mortality following allogeneic hematopoietic stem-cell transplantation (HSCT). For patients who develop acute GVHD, high-dose systemic glucocorticoids remain the cornerstone of treatment. Prognosis remains poor, however, with only one-third of patients achieving durable

responses to initial corticosteroid treatment. New approaches to GVHD prevention and treatment are urgently needed to improve outcomes after transplantation. This issue of *Bone and Marrow Transplantation Reviews* examines new insights into the underlying pathophysiology of GVHD development, with a focus on potential biomarkers and targets for therapeutic intervention.

## Antigen Presentation in Acute GVHD



Motoko Koyama, MD, PhD

The recognition of recipient alloantigen by donor T cells drives the development of GVHD. Therefore, another emerging approach for preventing GVHD following allogeneic HSCT involves interrupting the underlying process by which recipient alloantigen is presented to donor T cells. Recent research developments provide important insight into the understanding of alloantigen presentation and its role in therapeutic effects (i.e., graft-versus-leukemia) and adverse events (i.e., GVHD) following transplant.

### Alloantigen Presentation and GVHD

Both recipient and donor antigen-presenting cells (APCs) contribute to the induction and enhancement of GVHD following allogeneic HSCT [1]. After being infused, naïve donor T cells within the stem cell graft migrate to the recipient lymph nodes and/or other target organs, including the GI tract. Next, the naïve T cells encounter recipient alloantigen presented by recipient hematopoietic APCs and/or nonhematopoietic APCs (in the lymph nodes and GVHD target tissues). The recipient nonhematopoietic APCs, putatively including fibroblasts, endothelial cells and epithelial cells, obtain antigen-presenting functional capacity following tissue damage induced by conditioning chemotherapy and radiation.

Next, the donor T cells proliferate and differentiate into T helper 1 (Th1) or T helper 17 (Th17) cells. The Th1 and Th17 cells then induce GVHD in the recipient target tissues, especially the intestinal tract. Once tissue damage is initiated in the colon, the microbiome generates damage-associated molecular pattern/pathogen-associated

molecular pattern (DAMP/PAMP) signals that activate a subset of the donor CD103+ dendritic cells (DCs) to migrate from the colon to the mesenteric lymph node. The CC chemokine receptor 7 (CCR7) signaling pathway appears to mediate the migration of donor CD103+ DCs. Once within the mesenteric lymph node, the donor DCs present alloantigen to donor T cells and release IL-12, triggering further pathological differentiation and proliferation of Th1 and Th17 cells. In addition, those antigen-presented T cells are imprinted with gut-homing alpha-4-beta-7 integrins, leading to migration back to the GI tract and severe GVHD. Therefore, once GVHD is initiated by recipient APCs, a feed-forward loop involving antigen presentation by donor DCs exacerbates the severity of GVHD.

### Antigen Presentation by MHC class I and II

Several studies have examined the relationship between direct versus indirect antigen presentation and the initiation and maintenance of GVHD. In cases of direct antigen presentation, donor T cells recognize peptide-major histocompatibility complex (MHC) complexes on host APCs. By comparison, in cases of indirect antigen presentation, donor T cells recognize recipient-derived antigens (allo-antigens) loaded in the MHC of donor APCs.

In MHC-mismatched donors and recipients, the targets of GVHD are the recipient MHCs loading self-peptides [38]. The minor histocompatibility antigens (mHAs) also play a role in MHC-matched and MHC-mismatched donor-recipient pairs. The mHAs are peptides produced by polymorphic genes that differ between donor and recipient, which are then presented by MHCs. The mHAs are presented within both MHC class I and class II. In allogeneic HSCT recipients, MHC class I and MHC class II are differentially expressed. Indeed, mismatches at HLA class I and II significantly increase the risk of severe GVHD and transplant-related mortality [8]. In allogeneic HSCT recipients, MHC class I and MHC class II are differentially

expressed. Indeed, mismatches at HLA class I and II significantly increase the risk of severe GVHD and transplant-related mortality [8].

To better understand the antigen-presentation mechanisms underlying GVHD, 2 experimental models have been developed. The models propose that CD8+ T cells mediate MHC class I-dependent GVHD, whereas CD4+ T cells mediate class II-dependent GVHD. Within these models, the origin of the antigen and the process of antigen presentation differ. In a series of studies, researchers identified key characteristics of the cell types involved in each model:

- CD8+ T-cell-mediated GVHD: Recipient hematopoietic APCs are necessary and sufficient to initiate CD8+ T-cell-mediated GVHD [2]. To maximize the severity of CD8+ T-cell-mediated GVHD, however, donor APCs are also necessary [3].
- CD4+ T-cell-mediated GVHD: Recipient hematopoietic APCs are sufficient to elicit CD4+ T-cell-mediated GVHD [4,5].
- Recipient hematopoietic APCs: Additional research also confirmed that recipient DCs are sufficient to induce CD8+ T-cell-mediated GVHD and CD4+ T-cell-mediated GVHD [6,7].

Together, these findings indicate that recipient hematopoietic APC expressing MHC class I and II are involved in the development and progression of GVHD. However, it remains unclear whether recipient hematopoietic APCs are necessary for the induction of CD4+ T cell-mediated GVHD, that is, their absence reduces GVHD.

### Recipient Nonhematopoietic Antigen-Presenting Cells

In 2012, Koyama and colleagues demonstrated recipient nonhematopoietic APCs are sufficient to induce lethal acute GVHD by MHC class II antigen presentation [9]. The research team developed a mouse model of HSCT to characterize the presentation of recipient peptides within MHC class

II molecules. Although donor APCs were potentially able to induce lethal acute GVHD via MHC class II in the mouse model, recipient APCs were 100-fold to 1,000-fold more potent in inducing lethal acute GVHD.

The team also showed that the inflammatory environment following myeloablative conditioning enables T-cell activation in the lymphoid tissue independent of alloantigen. As a result, the T cells acquired a memory phenotype and gained access to targeted tissues. Therefore, the activated nonhematopoietic APCs generated potent allo-specific responses, leading to the expansion of alloreactive donor T cells within the GI tract and the production of inflammatory cytokines [9].

#### *Donor Alloantigen Presentation*

Donor APCs are not required to initiate GVHD, and in fact are inefficient at doing so in isolation [9]. However, these cells are required for maximal disease severity [3]. In 2009, Markey and colleagues examined the relative contribution of different populations of donor APCs involved in alloantigen presentation following allogeneic HSCT [10]. Donor conventional dendritic cells (cDCs) are the most potent APCs capable of presenting alloantigen.

By comparison, other cell types, including donor macrophages and plasmacytoid DCs, did not make a meaningful contribution to alloantigen presentation. Furthermore, the absence of donor B cells correlated with enhanced alloantigen presentation. These findings suggest a potential therapeutic role for cDC depletion and B-cell reconstitution to regulate alloreactivity following HSCT [10].

During GVHD, the mesenteric lymph nodes are the major site of donor alloantigen presentation. This process begins with the initiation of GVHD, which leads to the expansion and activation of CD103+ donor DC subsets in the colon. In the presence of CCR7, donor CD103+ DCs migrate from the colon to the mesenteric lymph nodes, where donor macrophages are dispensable for alloantigen presentation. Multiple signaling pathways are involved in coordinating this process, suggesting a range of opportunities for therapeutic intervention. The presence of CCR7-dependent DCs and antigen presentation are required to imprint donor T cells with the alpha-4-beta-7 integrin. Conversely, the absence of CCR7 is associated with reduced T cell expansion [11]. In addition, the DAMP/PAMP, Toll-like receptor

(TLR), and receptor for advanced glycation end products (RAGE) signaling pathways appear to be critical for promoting GVHD lethality [1].

#### **Summary**

Host nonhematopoietic cells are crucial for the induction of CD4+ T cell-dependent GVHD. Researchers are focusing on targeting the mechanisms of antigen presentation utilized by recipient nonhematopoietic APCs for the prevention of GVHD. Ongoing research involves characterizing additional cell types that function as recipient nonhematopoietic APCs, and elucidating the intracellular pathways that mediate antigen presentation.

Donor-derived colonic DCs appear to determine the severity of GVHD. Given that GVHD target organs do not harbor leukemic cells, and that donor DCs do not mediate the graft-versus-leukemia effects, strategies aimed at preventing GVHD should spare GVL. In the future, biomarkers may play an important role in identifying patients at high risk for severe GVHD who may benefit from preemptive treatment. Targeted therapies that disrupt the activity of key GVHD mediators (e.g., CCR7, IL-12, IL-6, and alpha-4-beta-7) will curtail the progression of tissue damage that leads to severe GVHD.

## **The Role of the Microbiome in GVHD**



*Ernst Holler, MD, PhD*

Manipulating the microbiome with decontamination and antibiotic prophylaxis in the setting of allogeneic HSCT are not new ideas. In 1974, investigators demonstrated that prolonged germ-free conditions prevented the development of GVHD following transplant in mice [13]. Overall

survival was 100% among mice kept in germ-free conditions for 100 days after HSCT, but dropped to 65% for animals maintained in a decontaminated environment until day 26. By comparison, 100% of mice died from GVHD when the germ-free conditions were maintained only until posttransplant day 8 [13].

In 2006, Leibovici and colleagues described the clinical benefits of antibiotic prophylaxis in a meta-analysis of randomized clinical trials conducted between 1980 and 2005 [14]. Among 1530 patients with acute leukemia treated with bone marrow transplantation, quinolone prophylaxis reduced all-cause mortality by 33% (relative risk [RR], 0.67; 95% confidence interval [CI], 0.46-0.98) and reduced the risk of

febrile episodes by 22% (RR 0.78; 95% CI, 0.74-0.83). In current clinical practice, many centers still use decontamination and prophylaxis as their cornerstone strategies to prevent infections and GVHD in patients undergoing transplantation.

#### **Human Microbiome Project: Understanding Microbiome Diversity**

The Human Microbiome Project (HMP), an initiative of the National Institutes of Health (NIH) designed to characterize the diversity of microorganisms found in health and disease human tissue, has provided important insights into the complexity of the gut microbiome.

One of the challenges of studying the human microbiome is that only 30% is

culturable. Additional high-throughput technologies, such as 16s ribosomal (rRNA) sequencing, have been used for culture-independent characterization of the human microbiome. With these techniques, researchers described large populations of commensal anaerobic bacteria (e.g., Bacteroidetes and Clostridium sp) involved in protection from inflammatory and autoimmune diseases. Overall, estimates from the HMP suggest that each human consists of approximately 30 trillion human cells, 100 trillion microbial cells (the microbiota), and 1.5 million active microbial metabolites.

#### *Early Loss of Microbiome Diversity*

Recent studies utilizing 16s rRNA analysis have characterized the relationship between intestinal microbiome diversity and GVHD. In 2012, Jeng and colleagues described changes in the intestinal microbiome associated with GVHD in both mice and humans [15]. GVHD correlated with specific shifts in flora—including a loss of Clostridiales and an expansion in Lactobacillales—and resulted in an overall loss in microbiome diversity. In mouse models of GVHD, pretransplant prophylaxis with broad-spectrum antibiotics induced a dramatic shift in microbiome diversity. In addition, antibiotic treatment before HSCT was associated with increased intestinal GVHD severity and worse GVHD survival.

In 2014, Holler and colleagues confirmed the association between antibiotic prophylaxis and increased intestinal GVHD severity in patients undergoing allogeneic HSCT [16]. The prospective study included 31 patients with hematologic cancers undergoing allogeneic SCT. All patients received antibiotic prophylaxis (trimethoprim plus sulfamethoxazole) beginning at hospital admission until day -1 before transplant, followed by ciprofloxacin plus metronidazole from day 0 until engraftment. Therapeutic antibiotics were also used in patients who developed infections. Stool specimens were collected from all patients before and after transplant, and genomic analysis of the stool microbiome

was used to monitor changes in the intestinal microflora throughout treatment.

Results from the 16S rRNA gene sequencing analysis showed an early loss of commensal bacteria (e.g., Clostridia) and a shift toward increased enterococcal flora (*E. faecium* and *E. faecalis*) in all patients, reflecting the use of prophylactic and therapeutic antibiotics. The shift toward enterococci was especially pronounced among patients who developed GI GVHD. After allogeneic HSCT, the mean proportion of enterococci was 21% in the stool samples of patients who did not develop GI GVHD. By comparison, the post-transplant proportion of enterococci was 46% in patients who subsequently developed GI GVHD, and increased to 74% during active GI GVHD.

#### *Long-Term Effects of Microbiome Diversity Loss*

Additional studies have also shown that a loss of intestinal microbiome diversity has adverse implications for long-term outcomes in patients undergoing allogeneic HSCT [17,18]. In 2015, Jenq and colleagues evaluated the intestinal microbiota of 62 patients 12 days after transplant [18]. Based on an analysis of stool samples, the degree of bacterial diversity was quantified using the standard inverse Simpson index. Patients were then categorized into 3 groups: low diversity (inverse Simpson index < 2; n = 34), medium diversity (inverse Simpson index 2-4; n = 20), and high diversity (inverse Simpson index >4; n = 26).

During the first 3 years following engraftment, there was a significant correlation between increased intestinal microbial diversity and improved overall survival (P = .019). Bacteria from the genus *Blautia* was found to correlate most strongly with reduced GVHD-related mortality. Patients with a higher proportion of *Blautia* on posttransplant day 12 demonstrated a significantly reduced risk of acute GVHD that required systemic steroid treatment or was steroid refractory (P < .01), reduced GVHD-related mortality (P = .004), and improved overall survival (P < .001). These findings underscore the central role of intestinal bacteria diversity in reducing adverse outcomes

following HSCT, and highlights the importance of specific bacterial populations [17,18].

#### *Urinary 3-Indoxyl Sulfate (3-IS): An Early Biomarker of Microbial Diversity*

Urinary 3-indoxyl sulfate (3-IS) is produced as a byproduct of the degradation of tryptophan to indole by intestinal commensal bacteria (e.g., Clostridiales), followed by the microsomal oxidation of indole to indoxyl and sulfonation in the liver. High urinary 3-IS levels (6.9  $\mu\text{mol}/\text{mmol}$  urinary creatinine) correlate with a high number of intestinal commensals and a high degree of microbial diversity, whereas low urinary 3-IS levels  $\leq 6.9 \mu\text{mol}/\text{mmol}$  urinary creatinine indicate few commensals and low overall diversity [16,19].

In 2015, Weber and colleagues demonstrated that urinary 3-IS levels measured immediately after allogeneic HSCT predict clinical outcomes [19]. The study included 131 patients undergoing allogeneic HSCT for the treatment of hematologic malignancies (n = 111), myelodysplastic syndrome (n = 17), or aplastic anemia (n = 3). Urinary 3-IS levels were measured weekly during the first 28 days following transplantation. Results showed that low 3-IS levels within the first 10 days of ASCT significantly predicted higher transplant-related mortality (P = .017) and decreased overall survival (P = .05) after 1 year. By comparison, classical risk factors such as patient age, disease stage, donor type, conditioning regimen, duration of antibiotic therapy, and duration of neutropenia did not correlate with the risk of transplant-related mortality after 1 year.

Next, investigators evaluated potential risk factors for early suppression of urinary 3-IS levels following allogeneic HSCT. In a multivariate analysis, early use of antibiotic treatment (P = .001), type of GI decontamination (P = .01), and NOD2/CARD15 (P = .04) genotype significantly correlated with low urinary 3-IS levels in the first 10 days following transplant [19]. Previous studies indicate that NOD2/CARD15 variants regulate the production of antimicrobial peptides in Paneth cells

**Table 1. Outcomes Following Prophylaxis with Ciprofloxacin/Metronidazole or Rifaximin [21]**

Posttransplant Endpoint	Ciprofloxacin/Metronidazole (n = 131)	Rifaximin (n = 90)	P Value
Enterococcal positivity	21.9%	6.9%	.05
Mean urinary 3-IS, day 0-10	7.3 $\mu\text{mol}/\text{mmol}$ creatinine	14.9 $\mu\text{mol}/\text{mmol}$ creatinine	.004
3-year NRM	39%	19%	.02

3-IS, 3-indoxyl sulfate; NRM, nonrelapse mortality.

**Table 2. Timing of Therapeutic Antibiotics After Transplant and Transplant Outcomes [22]**

	No Antibiotics (n = 88)	Early Antibiotics (n = 236)	Late Antibiotics (n = 297)
Treatment-related mortality	7%	34%*	21%
Overall survival	78%	51%†	67%

\*P = .001 versus late antibiotics and P &lt; .001 versus no antibiotics.

†P &lt; .001 versus late antibiotics and P = .001 versus no antibiotics.

**Table 3. Timing of Antibiotics and Markers of Intestinal Microbiome Diversity [22]**

	No Antibiotics (n = 88)	Early Antibiotics (n = 236)	Late Antibiotics (n = 297)
<b>Urinary 3-IS levels, <math>\mu\text{mol}/\text{mmol}</math> creatinine</b>			
Baseline	19.0	30.3	24.9
Days 0-28	11.1	5.5*	9.9

3-IS, 3-indoxyl sulfate.

\*P &lt; .001 versus late antibiotics and P = .02 versus no antibiotics.

and neutrophils. Accordingly, a deficiency in NOD2/CARD15 may facilitate adverse shifts in microbial diversity, leading to increased GI inflammation [20]. Together, these findings support the role of urinary 3-IS levels as a feasible biomarker of microbiome changes following allogeneic HSCT in the clinical setting.

### Prophylactic Antibiotics and Microbial Diversity

In a 2016 study, Weber and colleagues described the effects of different prophylactic antibiotic regimens on intestinal microbiome diversity and clinical outcomes [21]. The retrospective study included 394 patients who underwent allogeneic HSCT between 2008 and 2015. Between 2008 and 2012, all patients (n = 131) were treated with ciprofloxacin/metronidazole prophylaxis prior to transplant. Following a change in institutional protocol in 2012, all subsequent patients (n = 90) were switched to rifaximin

prophylaxis. During the first 28 days following transplant, all patients underwent weekly monitoring in urinary 3-IS levels and intestinal enterococcal load.

Compared with ciprofloxacin/metronidazole, rifaximin was associated with lower enterococcal positivity and higher urinary 3-IS concentrations following allogeneic ASCT (Table 1). Rifaximin was also associated with a lower risk of transplant-related mortality after 1 year (P = .04), a lower risk of NRM after 3 years (P = .02), and improved overall survival (P = .008) [21]. These results highlight the role of the antibiotic prophylaxis regimen in maintaining microbial diversity and improving patient outcomes following transplantation.

### Therapeutic Antibiotics and Microbial Diversity

More recently, Weber and colleagues have examined the interactions between therapeutic antibiotics following transplant,

intestinal microbiota, and clinical outcomes [22]. The retrospective analysis included 621 who underwent allogeneic ASCT at the University Hospital in Regensburg, Germany (n = 380) and the Memorial Sloan Kettering Cancer Center in New York, New York (n = 241) between 2008 and 2015. As described in the previous study, patients received antibiotic prophylaxis with ciprofloxacin/metronidazole (n = 189) until 2012, when the prophylaxis regimen was switched to rifaximin (n = 191).

Beyond the prophylactic regimens, patients received additional broad-spectrum antibiotic treatment with piperacillin/tazobactam as empiric first-line treatment for neutropenic fever and/or infections. Additional second-line regimens varied by treatment center. Patients were stratified into 1 of 3 groups according to the timing of therapeutic antibiotic therapy:

- No therapeutic antibiotics: 14%
- Early therapeutic antibiotics (days -7 to 0): 38%
- Late therapeutic antibiotics: (day 0 or after): 48%

Results showed that the early use of systemic antibiotics following allogeneic HSCT significantly increased the risk of treatment-related mortality compared with late antibiotic use (P = .001) or no antibiotic use (P = .005) (Table 2). Early antibiotic use was also associated with a decrease in the abundance of fecal commensal Clostridiales (P = .03) and lower urinary 3-IS levels (P < .001) compared with late antibiotic exposure (Table 3). In a multivariate analysis, early antibiotic use remained a significant predictor of transplant outcomes irrespective of traditional risk factors, including Karnofsky performance status, donor type, and underlying disease stage [22].

Additional studies confirm the association between systemic antibiotic use and changes in intestinal microbiome composition following transplantation [23,24]. In 2016, Routy and colleagues showed that the risk of grade II-IV acute



GVHD was significantly increased among patients who underwent gut decontamination with systemic antibiotics prior to allogeneic HSCT compared with those who did not (42% versus 28%, respectively;  $P < .001$ ) [23]. The difference was driven primarily by a 2-fold increase in the risk of GI GVHD among those who received antibiotics compared with those who did not (20.7% versus 10.8%, respectively;  $P = .003$ ). In 2017, Simms-Waldrip and colleagues described patterns in antibiotic-induced gut microbiota changes among pediatric patients undergoing allogeneic HSCT [24]. In this cohort, acute GVHD was strongly associated with cumulative antibiotic exposure—particularly exposure to antianerobic antibiotics (e.g., clindamycin)—as well as the depletion of commensal anaerobes such as Clostridiales [24].

### Mechanisms of Microbial Diversity Loss

Understanding the mechanisms of microbial diversity loss is critical for the development of effective strategies to prevent and treat GVHD. Endogenous tryptophan metabolites such as indoles appear to play a leading role in intestinal immune homeostasis [25]. In animal studies, indoles exert anti-inflammatory effects at the T-cell level. With the introduction of tryptophan as an energy source, lactobacilli populations expand and produce an aryl hydrocarbon receptor (AhR) ligand that facilitates the production of IL-22 via AhR-dependent IL-22 transcription. Therefore, by inducing the production of the anti-inflammatory IL-22 cytokine, indoles support the survival of mixed microbial communities in part by strengthening the mucosal-barrier properties of epithelial cells [25].

Another microbial metabolite-related mechanism of protection against epithelial cell damage in the gut following allogeneic HSCT involves the development of short-chain fatty acids (SCFAs) such as butyrate

[26]. In laboratory models of GVHD, the amount of butyrate in intestinal tissue is markedly reduced after transplantation, leading to decreased histone acetylation. Restoring butyrate improved the junctional integrity of intestinal epithelial cells, reduced the rate of epithelial cell apoptosis, and reduced the risk of GVHD. In addition, microbial metabolite-derived SCFAs appear to exert anti-inflammatory effects via the downregulation of IL-12, mediation of costimulatory molecules, and induction of regulatory T-cells.

The research team conducted additional experiments with 17 rationally selected strains of Clostridia known to produce high levels of butyrate. In a mouse model of GVHD, administering the 17-strain cocktail via gastric lavage before and after allogeneic HSCT significantly altered the intestinal microbiota to favor high butyrate-producing Clostridia. Compared with control animals that underwent HSCT without the 17-strain cocktail, animals that received the gastric lavage had significantly increased intestinal butyrate levels and a significantly reduced risk of GVHD. This suggests a possible therapeutic approach to altering intestinal microbiota to reduce the risk and severity of GVHD following transplantation [26].

In patients with acute GVHD, damage to Paneth cells appears to exacerbate the suppression of intestinal microbiota diversity. In 2013, Levine and colleagues

examined the number of Paneth cells in 116 duodenal biopsies obtained from patients with GI GVHD [27]. Patients with lower numbers of duodenal Paneth cells at the time of diagnosis were significantly more likely to have more severe GI GVHD ( $P < .0001$ ) and significantly less likely to respond to standard GVHD treatment ( $P < .0001$ ) than patients with more abundant Paneth cell numbers. Using light microscopy, the presence of 4 Paneth cells per high-powered field defined the threshold for stratifying patients into low-risk ( $\geq 4$  cells) and high-risk ( $< 4$  cells) groups. The cumulative rate of nonrelapse mortality at 6 months was more than 2-fold higher among patients in the high-risk group compared with those in the low-risk group (55% versus 23%, respectively;  $P < .0001$ ).

### Options for Altering Host-Microbiota Interactions

Taken together, preclinical and clinical evidence indicates that GVHD results from a disturbed balance in microbiota and a disturbed balance in the cells regulated by microbiota. The complexity of mechanisms involved in the development and progression of GVHD suggests many potential therapeutic targets. Options for manipulating the host-microbiota interactions include antibiotic, prebiotic, probiotic, and postbiotic modalities (Table 4) [28].

**Table 4. Potential Approaches to Manipulating Host-Microbiota Interactions [12,28]**

<b>Antibiotics</b>	<ul style="list-style-type: none"> <li>• Decontamination</li> <li>• Rifaximin</li> <li>• Timing of prophylactic and therapeutic antibiotics</li> <li>• Commensal-sparing antibiotics</li> <li>• Oral antibodies against intestinal pathogens (e.g., chicken IgY)</li> </ul>
<b>Prebiotics</b>	<ul style="list-style-type: none"> <li>• Non-digestible carbohydrates</li> <li>• Avoidance or encouragement of certain foods</li> </ul>
<b>Probiotics</b>	<ul style="list-style-type: none"> <li>• Fecal microbial transplant</li> <li>• Engineered microbes</li> <li>• Rationally selected strains (e.g., 17-strain Clostridia cocktail)</li> </ul>
<b>Postbiotics</b>	<ul style="list-style-type: none"> <li>• Short-chain fatty acids (e.g., butyrate)</li> <li>• Indoles and indole derivatives</li> <li>• Avoidance of foods compromising intestinal mucus barrier</li> </ul>

IgY, immunoglobulin yolk.

### Fecal Microbiota Transplantation in Refractory GVHD

Fecal microbiota transplantation (FMT) is emerging as a novel treatment option for restoring commensal microbes in patients with acute GVHD [29,30]. In a pilot study of the safety and efficacy of FMT, 4 patients with steroid-resistant ( $n = 3$ ) or steroid dependent ( $n = 1$ ) acute GI GVHD received FMT from healthy related donors [29]. The procedure was safe, with no patients experiencing any severe adverse events related to FMT. All patients responded to treatment, including 3 complete responses and 1 partial response, allowing for a reduction in concomitant steroid doses. An analysis of stool specimens before and after FMT showed a restoration of commensal microbiota [29].

Another pilot study examined the role of repeated FMT in 3 patients with severe cases of treatment-refractory acute GI GVHD following allogeneic HSCT [30]. All patients responded clinically with reduced stool volumes that normalized after repeated FMT from healthy donors. The FBMT procedure was safe, with no infectious complications [30].

### Additional Insights into Microbiota Diversity

Recent research has focused on the interactions between intestinal microbiota diversity and other clinical endpoints among patients undergoing treatment with allogeneic HSCT. Loss of diversity due to concomitant antibiotic treatment may reduce the efficacy of cytotoxic drugs and checkpoint inhibitors [31-33]. In 2016, Pflug and colleagues evaluated the interaction between antibiotics and antineoplastic treatment in patients with chronic lymphocytic leukemia or lymphoma enrolled in the CLL8 trial ( $n = 800$ ) and the Cologne Cohort of Neutropenic Patients trial ( $n = 122$ ), respectively [33]. The analysis focused on

**Table 5. Antibiotic Use and Response to Antineoplastic Therapy [33]**

Trial/Endpoint	Anti-Gram-Positive Antibiotic Use		P Value
	Yes	No	
<b>CLL8 Trial</b>	(n = 45)	(n = 755)	
Overall response rate	74.3%	90.2%	.007
Median PFS	14.1 months	44.1 months	< .001
Median OS	56.1 months	91.7 months	< .001
<b>Cologne Cohort of Neutropenic Patients</b>	(n = 21)	(n = 101)	
Overall response rate	42.9%	70.3%	.016
Median PFS	2.3 months	11.5 months	.001
Median OS	5.6 months	96.8 months	< .001

OS, overall survival; PFS, progression-free survival.

antibiotics with primary activity against Gram-positive bacteria.

In the cohort of CLL patients undergoing treatment with cyclophosphamide, concomitant use of anti-Gram-positive antibiotics was associated with a lower overall response rate ( $P = .007$ ), reduced progression-free survival ( $P < .001$ ), and worse overall survival ( $P < .001$ ) (Table 5). In a multivariate analysis, anti-Gram-positive antibiotic use was associated with a 2-fold increase in the risk of progression (HR, 2.090;  $P = .0001$ ) and a 3-fold increase in the risk of all-cause mortality (HR, 2.966;  $P < .0001$ ) [33].

Similarly, anti-Gram-positive antibiotic use was associated with a significantly lower overall response rate ( $P = .016$ ), reduced progression-free survival ( $P = .001$ ), and worse overall survival in the cohort of patients with relapsed lymphoma who were undergoing treatment with cisplatin (Table 11). The multivariate analysis showed that anti-Gram-positive antibiotic use increased the risk of all-cause mortality nearly 8-fold among patients with relapsed lymphoma (HR, 7.831;  $P < .0001$ ) [33].

The absence of certain commensal species in the GI tract is associated with an increased risk of relapse and disease progression following allogeneic HSCT [34]. In 2017, Peled and colleagues evaluated intestinal microbiota diversity among 541 patients undergoing treatment with allogeneic HSCT. Results

showed that a higher abundance of *Eubacterium limosum* significantly correlated with a decreased risk of disease progression. Each 10-fold increase in bacterial abundance was associated with an 18% decrease in the risk of relapse (HR, 0.82 per 10-fold increase in abundance;  $P = .009$ ). The 2-year cumulative risk of relapse or disease progression was 19.8% for patients who harbored *Eubacterium limosum*, compared with 33.8% among patients without this bacterial group (HR, 0.52;  $P = .01$ ). These findings suggest a potential role for certain bacterial species as prognostic biomarkers, as well as potential targets for therapeutic intervention.

### Summary

Maintaining the diversity of intestinal microbiota at the time of allogeneic HSCT is critical for improving transplant outcomes. Multiple studies have established the relationship between decreased microbiota diversity at the time of engraftment and increased risk of acute GVHD. Additional recent research highlights the potential interaction between intestinal microbiota and other treatment endpoints, including therapeutic response and risk of relapse. In summary, as a potent mediator of systemic immune responses, including anti-tumor responses, the intestinal microbiota is an important treatment target in the transplant setting.

## Acute GVHD: Biomarkers and Biology



James Ferrara, MD, DSc

The current first-line treatment approach to acute GVHD—high-dose systemic glucocorticoid therapy—has not changed in 40 years. Yet two-thirds of patients do not achieve durable responses to initial therapy, and survival in this group is poor. One of the challenges to better first-line therapy involves the lack of robust risk-stratification systems to facilitate individualized treatment plans. Instead, most patients are given “standard” therapy with high-dose corticosteroids. This uniform approach results in undertreatment for some patients, and unnecessary side effects related to overtreatment in others.

Within current GVHD grading systems, the risk of NRM correlates with maximal disease severity, which is designed retrospectively after the response to treatment is known. By comparison, clinical severity at diagnosis does not predict NRM. In one cohort of 300 patients, the NRM rate 12 months post-GVHD onset was 20%, 25%, and 34% among those who initially presented with grade I, grade II, and grade III/IV acute GVHD. [35] A prognostic model based on information available at the time of diagnosis is necessary to guide treatment decision-making for patients with acute GVHD.

In 2015, Levine and colleagues, on behalf of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), developed a prognostic score for acute GVHD based on three validated biomarkers of GVHD severity [35]. The resulting biomarker signature includes serum levels of regenerating islet-derived 3-alpha (Reg3 $\alpha$ ), suppression of tumorigenicity 2 (ST2), and tumor necrosis factor receptor-1 (TNFR1).

**Table 6. BMT CTN Prognostic Score: Survival Outcomes by Ann Arbor Score at Diagnosis [35]**

Endpoint at 12 Months	Ann Arbor Score at Diagnosis			P Value	
	1	2	3	Grade 1 vs 2	Grade 2 vs 3
NRM	8%	27%	46%	.002	.002
OS	76%	57%	44%	.006	.024

BMT CTN, Blood and Marrow Transplant Clinical Trials Network; GVHD, graft-versus-host disease; NRM, nonrelapse mortality.

**Table 7. Ann Arbor Reclassification of GVHD Clinical Grade [35]**

Ann Arbor Reclassification	Glucksberg I (n = 51)	Glucksberg II (n = 182)	Glucksberg III/IV (n = 67)
Ann Arbor grade 1	24%	28%	24%
Ann Arbor grade 2	58%	55%	51%
Ann Arbor grade 3	18%	20%	25%
P value for trend in NRM across Ann Arbor grades	.0051	.0012	0.087

GVHD, graft-versus-host disease; NRM, nonrelapse mortality.

The BMT CTN research group developed the prognostic algorithm in a study of 492 patients with newly diagnosed acute GVHD who were randomly assigned to training (n = 328) and testing (n = 164) cohorts. After testing, the algorithm was validated in another cohort of 300 patients enrolled in BMT CTN trials of GVHD treatment. The scoring system, named for University of Michigan at Ann Arbor, utilized an algorithm with weighted scoring for each serum biomarker assessed at diagnosis. The resulting Ann Arbor scores of 1, 2, and 3, indicating increased GVHD severity, significantly predicted higher rates of NRM and worse OS at 12 months (Table 6) [35].

The Ann Arbor scores also significantly predicted the response to standard systemic corticosteroid treatment. At day 28, the complete or partial response rates for patients with Ann Arbor scores of 1, 2, and 3 at diagnosis were 81%, 68%, and 46% respectively (P < .0001 for all comparisons) [35].

Compared with the Glucksberg scoring, the Ann Arbor scoring system resulted in the reclassification of clinical grade for many patients. In the validation cohort, 51 patients had Glucksberg grade I GVHD at diagnosis, indicating a rash affecting <50% of the body surface area. Another 182 patients were assigned Glucksberg grade II, indicating more extensive skin rash plus diarrhea or

liver involvement. In addition, 67 patients were classified as Glucksberg grade III/IV at initial presentation, indicating severe diarrhea and other symptoms. Across each Glucksberg grade, the Ann Arbor staging system assigned approximately one-quarter of patients to the lowest-risk group, slightly more than one-half to the intermediate-risk group, and slightly less than one-quarter to the highest-risk group (Table 7) [35].

Focusing on the Glucksberg grade I subgroup, 24% remained a low risk according to Ann Arbor scoring (Ann Arbor 1). In contrast, 58% were reclassified to intermediate risk (Ann Arbor 2) and 18% were reclassified to high risk (Ann Arbor 3). Despite all patients in this subgroup sharing the Glucksberg grade classifications, the Ann Arbor scoring system was able to discriminate statistically significant differences in NRM risk among those reclassified to Ann Arbor scores 1, 2, and 3 (P = .0051 for trend). The Ann Arbor system similarly discriminated different levels of NRM risk among patients who were classified as having Glucksberg grade II (P = .0012) or Glucksberg grade III/IV (P = 0.087) GVHD based on presenting symptoms (Table 2) [35].

### The MAGIC Algorithm for Early GVHD

Among the GVHD biomarkers identified to date, ST2 demonstrates the strongest

correlation with early acute GVHD. The vast majority of soluble ST2 molecules are produced by stromal cells and endothelial cells in the gastrointestinal (GI) tract. Preclinical studies demonstrate that ST2 is released from activated T cells and acts as a decoy receptor for soluble interleukin (IL)-33. By comparison, REG3 $\alpha$  is produced by Paneth cells and stored in the mucus of the GI tract, where the large molecules are released as GVHD progresses. Additional cytokines and cell types, such as IL-22 and ILC3 cells, regulate the release and migration of REG3 $\alpha$ . As more is understood about the activity of ST2 and REG3 $\alpha$  in the pathophysiology of GVHD, these biomarkers are playing increasingly prominent roles in predictive algorithms.

In 2017, Hartwell and colleagues developed another biomarker-based predictive algorithm designed to identify patients who are at risk for life-threatening, steroid-resistant GVHD before the development of clinical symptoms [36]. To develop the algorithm, the research team evaluated patient samples and data from the Mount Sinai Acute GVHD International Consortium (MAGIC), representing investigators from Mount Sinai, the University of Michigan, Mayo Clinic, The Ohio State University, and the University of Pennsylvania, Emory University, and centers in Bangkok, Dresden, Hamburg, Regensburg, and Würzburg. The participating centers were selected with the goal of developing a predictive algorithm that would be applicable in patients and clinical settings worldwide.

Researchers hypothesized that a biomarker signature measured 7 days after transplant could be used to predict severe GVHD and 6-month NRM. In total, blood samples were obtained from 1,287 patients on day 7 after hematopoietic stem cell transplantation (HSCT) and prior to the onset of GVHD clinical symptoms. In the training cohort (n = 620), computer models compared 13 potential algorithms based on 4 GVHD biomarkers (ST2, REG3 $\alpha$ , TNFR1, and IL-2R $\alpha$ ) to identify

**Table 8. MAGIC Predictive Algorithm and Risk of 6-Month Nonrelapse Mortality [36]**

Cohort	Risk of NRM at 6 Months			P Value
	All Patients	Low Risk	High Risk	
Training set	n = 620	n = 520	n = 100	
	11%	7%	28%	< .001
Testing set	n = 309	n = 255	n = 54	
	12%	7%	33%	< .001
Validation set	n = 358	n = 286	n = 72	
	13%	10%	26%	< .001

MAGIC, Mount Sinai Acute GVHD International Consortium; NRM, nonrelapse mortality.

the most accurate prediction of NRM at 6 months. The final algorithm was applied to an independent test cohort (n = 309) and validation cohort (n = 358).

The computer models tested all possible combinations of biomarkers, including algorithms of all 4 biomarkers, or the combination of the 3 best biomarkers (ST2, REG3 $\alpha$ , IL-2R $\alpha$ ), 2 best biomarkers (ST2, REG3 $\alpha$ ), or best single biomarker (ST2). The final best predictive algorithm utilized 2 biomarkers, ST2 and REG3 $\alpha$ . Next, thresholds for discriminating between low-risk and high-risk were selected to achieve 3 goals: 1) generate a reliable probability of 6-month NRM for each patient; 2) identify the largest number of high-risk patients; and 3) identify the maximum difference in NRM risk between patients classified into the low-risk and high-risk groups.

Applying the 2-biomarker algorithm across the training, testing, and validation cohorts, 80% to 84% of patients were categorized as low-risk, and 16% to 20% of patients were classified as high-risk. The cumulative 6-month NRM rates was 11% to 13% for all patients in all cohorts. In each cohort, however, the algorithm identified statistically significant differences in the 6-month NRM rate between those classified as low risk (7% to 10%) and those classified as high risk (26% to 33%) (P < .001 for each cohort) (Table 8) [36]. The predictive algorithm also performed well in stratifying risk for additional clinical outcomes. In the validation cohort, the 6-month overall survival (OS) rate was 85% in the low-risk group and 68% in the high-risk group (P < .001) [36].

### MAGIC Algorithm Performance Across Patient Subgroups

Researchers also assessed the performance of the algorithm among patient subgroups defined by pretransplant risk factors (Table 9). Among patients who underwent a related-donor transplant (n = 517), 88% were classified as low risk and 12% were classified as high risk. The 6-month cumulative NRM rates were 5% and 26%, respectively. Among those who underwent unrelated-donor transplants, only 78% were classified as low risk, whereas 21% were classified as high risk. The 6-month NRM rates were 10% and 30% in the low-risk and high-risk groups, respectively [36].

Similar trends were observed across patient subgroups defined by other pretransplant risk factors, including donor type, age, use of anti-thymocyte globulin as GVHD prophylaxis, and intensity of conditioning regimen (Table 4). Overall, the algorithm consistently identified differences in 6-month NRM rates of approximately 20% between low-risk and high-risk group. In addition, patients with traditional pretransplant risk factors were more likely to be classified as high-risk according to the predictive algorithm [36].

The MAGIC algorithm also predicted significant differences in GVHD-specific outcomes between the low-risk versus high-risk groups, including:

- Acute GVHD-related mortality (4% vs 18%; P < .001)
- Steroid-refractory GVHD (15% vs 35%; P < .001)
- Severe GI GVHD (8% vs 17%; P < .001)

### MAGIC Algorithm Performance at GVHD Symptom Onset

Next, investigators evaluated the performance of the 2-biomarker algorithm based on plasma samples collected at the time of GVHD onset, rather than 7 days after transplantation. Within the MAGIC study population, plasma samples were available from 212 patients at the time of GVHD onset. As described in the previous section, the 3-biomarker Ann Arbor scoring system classified patients into low-risk, intermediate-risk, and high-risk groups based on serum biomarker concentrations collected at the time of symptom onset [36]. Researchers used the 2-biomarker MAGIC algorithm to define 3 district risk groups that approximated the same NRM rates as those identified by the 3-biomarker Ann Arbor scoring system (Table 10) [36].

Of note, many more patients were stratified into the low-risk group using the new 2-biomarker algorithm compared with the initial 3-biomarker algorithm (45% versus 17%, respectively). Some of the differences in algorithm performance between the 2015 and 2017 studies may be attributed to increased sensitivity of the commercially available ST2 biomarker assay [35,36].

### Ann Arbor Algorithm Performance After Starting GVHD Treatment

The 2-biomarker MAGIC algorithm also performs well when applied after patients have initiated treatment for GVHD. At the 2017 BMT Tandem annual meetings, Major-Monfried and colleagues presented data from a new study using serum biomarker concentrations collected 1 week after systemic corticosteroid treatment among 378 patients with acute GVHD [37]. Patients were separated into testing (n = 236) and validation (n = 142) cohorts. Based on results from the 3-biomarker panel (ST2, REG3 $\alpha$ , TNFR1), approximately 25% of all patients were classified as low-risk, and 75% were classified as high-risk (Table 11).

Additional results showed a significant correlation between the risk group assigned

after treatment initiation and GVHD treatment outcomes. Compared with patients in the high-risk group, those in the low-risk group were more likely to achieve a complete or partial response to treatment at 28 days (P < .0001). Patients in the low-risk group also had a lower risk of NRM at 6 months (P < .0001) and improved OS at

6 months (P < .0001) compared with high-risk patients (Table 6) [37].

In summary, these findings support the role of a biomarker-based algorithm to predict GVHD outcomes before the onset of GVHD symptoms, at the onset of symptoms, and after the initiation of treatment [35-37].

**Table 9. Risk of 6-Month Nonrelapse Mortality by Pretransplant Risk Factors [36]**

Risk Group	Risk of NRM at 6 Months		P Value
	Low Risk	High Risk	
Related donor (n = 517)	n = 454 5%	n = 63 26%	< .001
Unrelated donor (n = 770)	n = 607 10%	n = 163 30%	< .001
HLA-matched (n = 1059)	n = 884 7%	n = 175 26%	< .001
HLA-mismatched (n = 228)	n = 177 13%	n = 51 39%	< .001
Reduced-intensity conditioning (n = 548)	n = 476 8%	n = 72 37%	< .001
Full-intensity conditioning (n = 739)	n = 585 8%	n = 154 25%	< .001

HLA, human leukocyte antigen; NRM, nonrelapse mortality.

**Table 10. Algorithm Performance Applied at the Time of GVHD Onset [36]**

	Low Risk (AA1)	Intermediate Risk (AA2)	High Risk (AA3)
<b>2-Biomarker Algorithm (MAGIC)</b>			
Patients	45%	28%	27%
6-month NRM	8%	24%	46%
<b>3-Biomarker Algorithm (Ann Arbor)</b>			
Patients	17%	62%	21%
6-month NRM	47%	19%	8%

AA, Ann Arbor; GVHD, GVHD, graft-versus-host disease; NRM, nonrelapse mortality.

**Table 11. Algorithm Performance After Starting GVHD Treatment [37]**

Endpoint	Risk Group		P Value
	Low Risk	High Risk	
<b>Test cohort (n = 236)</b>	<b>(n = 178)</b>	<b>(n = 58)</b>	
6-month NRM	17%	57%	< .0001
6-month OS	72%	37%	< .0001
<b>Validation cohort (n = 142)</b>	<b>(n = 101)</b>	<b>(n = 41)</b>	
6-month NRM	14%	57%	< .0001
6-month OS	79%	38%	< .0001

GVHD, graft-versus-host disease; NRM, nonrelapse mortality; OS, overall survival.

## Emerging Insights into the Biology of GVHD

Recent clinical evidence suggests that patients with early GVHD experience a decrease of REG3 $\alpha$  levels in the GI epithelium and a corresponding increase in plasma REG3 $\alpha$  concentrations. In an experimental mouse model of acute GVHD, the same patterns of decreasing concentrations of REG3 $\gamma$  (the homologue of human REG3 $\alpha$ ) in the GI epithelium plus increasing REG3 $\gamma$  plasmas levels were reproduced. In animals without GVHD, immunohistochemistry shows that REG3 $\gamma$  is strongly upregulated in villi throughout the GI tract. By comparison, very little REG3 $\gamma$  is detected in the GI tract in animals with induced GVHD.

Experiments in REG3 $\gamma$ -knockout animals also highlight the central role of REG3 $\gamma$  in GVHD development and progression. Compared with REG3 $\gamma$ -wild-type mice, overall survival is dramatically decreased in REG3 $\gamma$ -knockout mice following HSCT. REG3 $\gamma$ -knockout mice also show a significant reduction in the number of Paneth cells immediately after HSCT,

although by posttransplant day 10 the population of Paneth cells in the GI tract has normalized in the absence of GVHD.

Additional animal studies demonstrate the role of IL-22 as a mediator of REG3 $\gamma$  levels in the GI epithelia. Inducing GVHD resulted in characteristic histologic changes (e.g., villus blunting) and the depletion of REG3 $\gamma$ . After inducing GVHD, however, administering exogenous IL-22 restored normal GI epithelial histology and triggered to a marked REG3 $\gamma$  upregulation. Furthermore, as REG3 $\gamma$  levels increased in the GI tract in response to IL-22 administration, there was a corresponding decrease in plasma REG3 $\gamma$  concentrations. Therefore, the REG3 $\gamma$  that leaks into the serum appears to be a surrogate marker for endothelial crypt damage. Additional experiments demonstrated that the presence of functional REG3 $\gamma$  is required to achieve IL-22-mediated GVHD rescue.

### Summary

In summary, the MAGIC research group has developed a 2-biomarker (ST2,

REG3 $\alpha$ ) algorithm that predicts NRM and other GVHD outcomes based on data from more than 1,000 patients representing 11 BMT centers across 3 continents. The predictive algorithm can be used at multiple time points to identify patients at increased risk of lethal GVHD: early (i.e., 7 days after HSCT), at the time of GVHD symptom onset, and after 1 week of GVHD treatment. Subgroup analyses show that the algorithm predicts GVHD outcomes independently of pretransplant risk factors, GVHD grade, or response to treatment. Given its generalizability, the biomarker-based predictive algorithm may play a role in guiding preemptive GVHD treatment, including treatments aimed at the GI tract.

New biologic insights provide a better understanding of the GVHD process, which involves ILC3 cell damage, reduced IL-22 levels, and increased REG3 $\alpha$  production. Emerging treatment options for acute GVHD may veer away from immunosuppression and focus instead on restoring the function of the innate immune system in the GI tract.

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1. Which serum biomarker most strongly correlates with early acute GVHD?
  - A. Interleukin (IL)-12
  - B. IL-22
  - C. Suppression of tumorigenicity 2 (ST2)
  - D. Tumor necrosis factor (TNF)-alpha
2. The 2-biomarker algorithm developed by the MAGIC research group predicts nonrelapse mortality following transplant when used at which time point?
  - A. Within 7 days of allogeneic HSCT
  - B. At the time of GVHD symptom onset
  - C. After 1 week of starting GVHD treatment
  - D. All of the above
3. Which of the following is associated with an increased risk of acute GVHD?
  - A. Late expansion of commensal bacteria in the GI tract
  - B. Increased diversity of intestinal microbiota
  - C. Early loss of commensal bacteria in the GI tract
  - D. All of the above
4. Higher urinary 3-indoxyl sulfate (3-IS) levels correlate with which of the following?
  - A. Higher degree of intestinal microbial diversity
  - B. Lower degree of intestinal microbial diversity
  - C. Higher risk for post-transplant neutropenia
  - D. Greater loss of intestinal Paneth cells
5. Compared with ciprofloxacin/metronidazole prophylaxis, rifaximin prophylaxis prior to allogeneic HSCT is associated with which of the following?
  - A. Increased duration of hospital stay following transplant
  - B. Decreased urinary 3-IS levels on days 0-7 following transplant
  - C. Increased risk of treatment-related mortality
  - D. Decreased risk of nonrelapse mortality