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Flares of acute graft-versus-host disease: a Mount Sinai Acute GVHD International Consortium analysis

Yu Akahoshi,[1,2] Nikolaos Spyrou,[1] Matthias Hoepting,[3] Paibel Aguayo-Hiraldo,[4] Francis Ayuk,[5] Chantiya Chanswangphuwana,[6] Hannah K. Choe,[7] Matthias Eder,[8] Aaron M. Etra,[1] Stephan A. Grupp,[9,10] Elizabeth O. Hexner,[11] William J. Hogan,[12] Carrie L. Kitko,[13] Sabrina Kraus,[14] Monzr M. Al Malki,[15] Pietro Merli,[16] Muna Qayed,[17] Ran Reshef,[18] Tal Schechter,[19] Evelyn Ullrich,[20] Ingrid Vasova,[21] Matthias Wölfl,[22] Robert Zeiser,[23] Janna Baez,[1] Rahnuma Beheshti,[1] Gilbert Eng,[1] Sigrun Gleich,[3] Stelios Kasikis,[1] Nikolaos Katsivelos,[1] Steven Kowalyk,[1] George Morales,[1] Rachel Young,[1] Zachariah DeFilipp,[24] James L. M. Ferrara,[1,*] John E. Levine,[1,*] and Ryotaro Nakamura[15,*]

[1]The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Division of Hematology, Jichi Medical University Saitama Medical Center, Saitama, Japan; [3]Department of Hematology and Oncology, Internal Medicine III, University of Regensburg, Regensburg, Germany; [4]Cancer and Blood Disease Institute, Children's Hospital Los Angeles, University of Southern California, Los Angeles, CA; [5]Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [6]Division of Hematology and Center of Excellence in Translational Hematology, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand; [7]Blood and Marrow Transplantation Program, The Ohio State University, Columbus, OH; [8]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany; [9]Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [10]Department of Pediatrics, and [11]Department of Medicine and Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [12]Division of Hematology, Mayo Clinic, Rochester, MN; [13]Pediatric Stem Cell Transplant Program, Vanderbilt University Medical Center, Nashville, TN; [14]Department of Internal Medicine II, University Hospital of Würzburg, Würzburg, Germany; [15]Department of Hematology/Hematopoietic Cell Transplantation, City of Hope, Duarte, CA; [16]Department of Pediatric Hematology/Oncology and of Cell and Gene Therapy, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [17]Division of Pediatric Hematology/Oncology and Bone Marrow Transplantation, Aflac Cancer and Blood Disorders Center, Emory University and Children's Healthcare of Atlanta, Atlanta, GA; [18]Blood and Marrow Transplantation Program and Columbia Center for Translational Immunology, Columbia University Irving Medical Center, New York, NY; [19]Division of Hematology/Oncology/BMT, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; [20]Department of Pediatrics, Experimental Immunology and Cell Therapy, Goethe University Frankfurt, Frankfurt, Germany; [21]Department of Internal Medicine 5, Hematology and Oncology, Friedrich-Alexander-Universität Erlangen-Nürnberg and University Hospital Erlangen, Erlangen, Germany; [22]Pediatric Blood and Marrow Transplantation Program, Children's Hospital, University Hospital of Würzburg, Würzburg, Germany; [23]Department of Medicine I, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany; and [24]Hematopoietic Cell Transplant and Cellular Therapy Program, Massachusetts General Hospital, Boston, MA

Key Points

- After initial clinical response, flares of acute GVHD are common and associated with higher NRM.
- MAGIC biomarkers at first CR/VGPR can predict GVHD flares.

The absence of a standardized definition for graft-versus-host disease (GVHD) flares and data on its clinical course are significant concerns. We retrospectively evaluated 968 patients across 23 Mount Sinai Acute GVHD International Consortium (MAGIC) transplant centers who achieved complete response (CR) or very good partial response (VGPR) within 4 weeks of treatment. The cumulative incidence of flares within 6 months was 22%, and flares were associated with a higher risk of nonrelapse mortality (NRM; adjusted hazard ratio [aHR], 4.84; 95% confidence interval [CI], 3.19-7.36; $P < .001$). Flares were more severe (grades 3/4, 41% vs 16%; $P < .001$) and had more frequent lower gastrointestinal (LGI) involvement (55% vs 32%; $P < .001$) than the initial GVHD. At CR/VGPR, elevated MAGIC biomarkers predicted the future occurrence of a flare, along with its severity and LGI involvement. In multivariate analyses, higher Ann Arbor (AA) biomarker scores at CR/VGPR were significant risk factors for flares (AA2 vs AA1: aHR, 1.81 [95% CI, 1.32-2.48; $P = .001$]; AA3 vs AA1: aHR, 3.14 [95% CI, 1.98-4.98; $P < .001$]), as were early response to initial treatment (aHR, 1.84; 95% CI, 1.21-2.80; $P = .004$) and HLA-mismatched unrelated donor (aHR, 1.74; 95% CI, 1.00-3.02; $P = .049$). MAGIC biomarkers also stratified the risk of NRM both at CR/VGPR and at the time of flare. We conclude that GVHD flares are common and carry a significant mortality risk. The occurrence of future flares can be predicted by serum biomarkers that may serve to guide adjustment and discontinuation of immunosuppression.

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*J.L.M.F., J.E.L., and R.N. contributed equally to this work.

Data will be available upon reasonable request from the corresponding author, John E. Levine (john.levine@mssm.edu) or Yu Akahoshi (akahoshiu@gmail.com).

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

Introduction

Acute graft-versus-host disease (GVHD) is a common life-threatening complication after allogeneic hematopoietic cell transplantation (HCT).[1,2] The current standard first-line treatment is systemic steroids, which can induce a clinical response in a majority of patients.[3-7] However, acute GVHD symptoms often recur (flare) after the tapering or discontinuation of steroids.[8] Indeed, in a recent randomized, phase 3 trial of ruxolitinib for steroid-refractory acute GVHD (REACH 2 trial), one-third of enrolled patients were eligible due to inability to taper systemic corticosteroids.[9] But to date, limited data are available regarding the incidence, clinical presentations, and outcomes of flares of acute GVHD. These fundamental knowledge gaps hinder the progress toward risk-adapted patient management including tapering of immunosuppressive agents.

In this analysis from the Mount Sinai Acute GVHD International Consortium (MAGIC), we first defined a flare of acute GVHD according to expert consensus in our consortium. Using this definition, we sought to characterize the incidence, clinical presentation, and long-term outcomes of flares in a large multicenter cohort with prospectively collected clinical and laboratory data. We also evaluate the risk factors for flares at the time of initial clinical response to treatment, with a focus on the ability of MAGIC serum biomarkers to predict long-term outcomes when clinical symptoms are minimal or absent.

Methods

Patient selection

The MAGIC database and biorepository collects detailed clinical information and blood samples from patients who undergo allogeneic transplants, at 23 international HCT centers in North America, Europe, and Asia. Patients are prospectively monitored for acute GVHD symptoms and treatment every week through day 100 after HCT and for 4 weeks after the initiation of systemic treatment and then less frequently up until 2 years after their HCT. GVHD symptoms are adjudicated according to a rigorous prospective-specimen-collection, retrospective-blinded-evaluation study design.[10-12] Informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Adult and pediatric patients who received their first HCT between 2014 and 2021 were included in this study if they achieved complete response (CR) or very good partial response (VGPR) to systemic steroid treatment for acute GVHD (at least methylprednisolone 0.1 mg/kg per day or equivalent), within 4 weeks without primary disease relapse, and if a serum sample was available at the first achievement of CR/VGPR. Responses were assessed without regard to treatment given for GVHD. Supplemental Table 1 shows the number of patients from each participating center, which includes 324 patients who were included in a prior analysis of biomarkers as a response end point.[13]

Definitions

Acute GVHD was diagnosed and staged according to the published MAGIC consensus criteria.[12] CR was defined as the complete resolution of acute GVHD manifestations. VGPR was defined as any improvement that approximates CR but has residual stage 1 skin disease.[14] Flares were defined as the earliest date that 2 criteria were both met: symptom severity increased by at least 1 stage in at least 1 organ and intensified treatment (methylprednisolone increased by at least 0.25 mg/kg or equivalent or addition of another systemic immunosuppressive agent). Flares after primary disease relapse, donor lymphocyte infusion or the onset of chronic GVHD[15] were not included in the analysis. HCT-specific comorbidity index (HCT-CI) scores and intensity of conditioning regimens were defined as per published criteria.[16,17] Death was considered related to acute GVHD if GVHD symptoms were present at the time of death or if death occurred from a complication such as infection while receiving systemic treatment for acute GVHD (≥10 mg methylprednisolone equivalent [MPE] per day).[18] The weekly steroid taper rate was calculated as described in the supplemental Methods, and patients were assigned to rapid and slow taper groups as follows: the slow taper group included patients who were tapered <20% per week if the maximum steroid dose was ≤1 mg/kg or <30% per week if the maximum steroid dose was >1mg/kg. All other patients were included in the rapid taper group.

Serum samples

Serial serum samples were prospectively collected, cryopreserved, and stored at a central laboratory. Serum concentrations of suppressor of tumorigenicity 2 (ST2)[19] and regenerating islet-derived protein 3-α (REG3α)[20] were analyzed by enzyme-linked immunosorbent assays, as previously reported.[21-23] The MAGIC algorithm probability (MAP) was calculated as a single value between 0.001 and 0.999 according to the formula: $\log(-\log[1 - \text{MAP}]) = -11.263 + 1.844(\log_{10}\text{ST2}) + 0.577(\log_{10}\text{REG3}\alpha)$ and validated thresholds for Ann Arbor (AA) scores were used ($\text{AA1} < 0.14$; $0.14 \leq \text{AA2} < 0.29$; $\text{AA3} \geq 0.29$).[18,21,22,24-26]

Statistical analysis

Grouped variables were compared using the Fisher exact test, and continuous variables were compared by the Mann-Whitney *U* test. The analyses of flare as a time-dependent covariate for the risk of nonrelapse mortality (NRM) used cause-specific Cox proportional hazards regression models.[27] The cumulative incidence of flare and NRM were estimated and plotted according to the Gray method. Predictors of flares were evaluated using the method of Fine and Gray in multivariate analysis. Competing risks for the cumulative incidence of flare were the relapse of primary and death without flare of GVHD, whereas the competing risk for NRM was relapse. All outcomes were censored at 6 months from the starting point (either at CR/VGPR or at the onset of flare).

We included the following variables as potential risk factors for GVHD flare in a multivariate analysis: maximum GVHD grade, maximum steroid dose before CR/VGPR, time to CR/VGPR, use of immunosuppressive agents other than steroids before CR/VGPR, response to treatment, and serum biomarkers MAP at CR/VGPR. We included additional covariates in the multivariate analysis based on their known prognostic significance on GVHD outcomes:[24,28,29] recipient age at HCT, sex mismatch, donor type, GVHD prophylaxis, HCT-CI, use of antithymocyte globulin or alemtuzumab, and conditioning regimen intensity. In the multivariate analysis which treated a flare as a time-dependent covariate for NRM, we reduced

the number of covariates because of the small number of events. These included maximum GVHD grade, maximum steroid dose before CR/VGPR, timing of achievement of CR/VGPR, use of immunosuppressive agents other than steroids before CR/VGPR, response to treatment, recipient age,[24] and HCT-CI.[29] Correlations among the variables in the multivariate models were tested to avoid multicollinearity (supplemental Table 2).[30]

The interaction between AA scores and steroid taper rate was measured using additive scales.[31] The additive scale interaction was evaluated by calculating the relative risk caused by interaction, and its confidence interval (CI) was estimated based on the delta method.[32]

Statistical significance was set at .05, and all *P* values were 2-tailed. Statistical analyses were performed with R or EZR version 1.61 (Jichi Medical University Saitama Medical Center, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 4.2.2, Vienna, Austria).[33]

Results

Patient characteristics and incidence of flares

The CONSORT diagram for this analysis shows that the MAGIC database included 1302 patients who received systemic corticosteroids for acute GVHD and who achieved CR/VGPR within 4 weeks (Figure 1). Serum samples at the time of CR/VGPR were not available in 334 patients. The 6 month NRM of the remaining 968 patients did not differ from the total group (10% vs 10%, $P = .889$). Because the cumulative incidence of flare and of NRM were similar after CR and VGPR in these 968 patients, we combined them as a single clinical response to first-line therapy (supplemental Figure 1). The median maximum daily dose of corticosteroids before CR/VGPR in these patients was 1 mg/kg

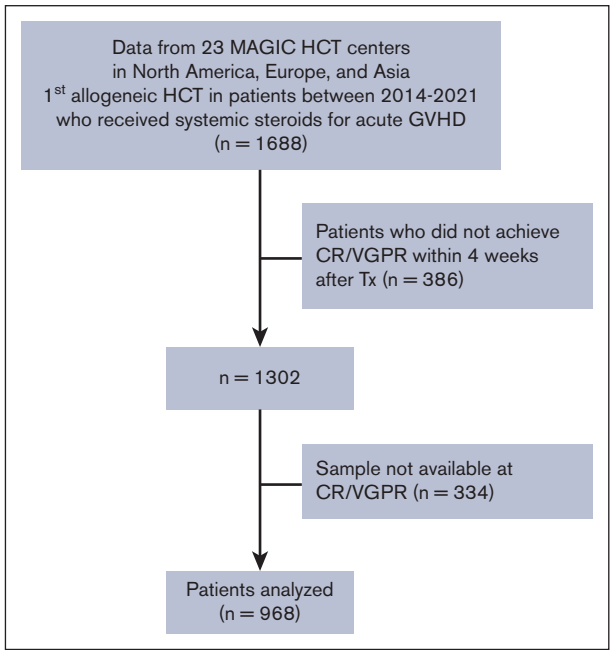

Figure 1. CONSORT diagram. Tx, treatments.

Table 1. Patient characteristics

	Overall
	n = 968 (%)
Median age at HCT, y (range)	55 (0-79)
Recipient age, y	
<18	106 (11)
18-54	377 (39)
≥55	485 (50)
Primary disease	
Acute leukemia	524 (54)
MDS/MPN	238 (25)
Lymphoma	85 (9)
Other	121 (13)
Sex mismatch	
Female to male	153 (16)
Others	815 (84)
Donor type	
HLA-matched related	172 (18)
HLA-matched unrelated	540 (56)
HLA-mismatched unrelated	104 (11)
Haploidentical	116 (12)
Umbilical cord blood	36 (4)
GVHD prophylaxis	
CNI based	738 (76)
PTCy based	188 (19)
Others	42 (4)
HCT-CI	
0-2	652 (67)
≥3	316 (33)
Use of ATG or alemtuzumab	
No	553 (57)
Yes	415 (43)
Donor source	
Bone marrow	202 (21)
Peripheral blood	730 (75)
Umbilical cord blood	36 (4)
Conditioning	
MAC (TBI <8 Gy)	421 (44)
MAC (TBI ≥8 Gy)	156 (16)
RIC	391 (40)
Median year of HCT (range)	2018 (2014-2021)
Max grades before CR/VGPR	
Grades 1-2	788 (81)
Grades 3-4	180 (19)
Timing of achievement of CR/VGPR	
Late response (>14 d)	172 (18)
Early response (≤14 d)	796 (82)

ATG, antithymocyte globulin; CNI, calcineurin inhibitor; MAC, myeloablative conditioning; MDS/MPN, myelodysplastic syndromes/myeloproliferative neoplasms; RIC, reduced intensity conditioning; TBI, total body irradiation.
*Excludes ruxolitinib.

Table 1 (continued)

	Overall
	n = 968 (%)
Max steroid dose before CR/VGPR	
<1 mg/kg	460 (48)
≥1 mg/kg	508 (53)
GVHD treatment before CR/VGPR	
Steroid alone	864 (89)
Steroid + ruxolitinib	22 (2.0)
Steroid + other*	82 (8.5)
Treatment response	
CR	682 (71)
VGPR	286 (30)

ATG, antithymocyte globulin; CNI, calcineurin inhibitor; MAC, myeloablative conditioning; MDS/MPN, myelodysplastic syndromes/myeloproliferative neoplasms; RIC, reduced intensity conditioning; TBI, total body irradiation.
*Excludes ruxolitinib.

methylprednisolone or equivalent (range, 0.1-3.2 mg/kg; Table 1; supplemental Table 3). Most patients were treated for maximum of grade 1/2 GVHD (81%) and achieved CR/VGPR (82%) within 2 weeks.

Association of flare with long-term outcomes

The overall cumulative incidence of NRM at 6 months after CR/VGPR was 10% (95% CI, 8-12), with a median follow-up of survivors after CR/VGPR of 22 months (range, 1-39). Follow-up was less than 6 months for 22 survivors (2%) after CR/VGPR. The 6 month cumulative incidence of flares after first CR/VGPR was 22% (95% CI, 19-24; Figure 2A). The median time to flare was 28 days (range, 2-448), and 87% of flares occurred within 3 months from CR/VGPR. Of the 210 patients experiencing flares, 138 (66%) were treated with an escalation of steroids, 47 (22%) received second-line agents, and 25 (12%) were treated with an increase of steroids in addition to second-line therapy. The cumulative incidence of 6-month NRM after flares was 27% (95% CI, 21-33). Six-month NRM was not significantly different for patients whose flares were treated by increasing steroid dose (23%; 95% CI, 16.0-30.0), adding a second-line agent (33%; 95% CI, 19.5-46.3), or both (40%; 95% CI, 20.8-58.6; $P = .086$). Patients with grade 3/4 GVHD at the time of flare, however, were more likely to experience NRM than patients with grade 1/2 GVHD (47% vs 12%; $P < .001$). The development of a flare treated as a time-dependent covariate was significantly associated with higher risk of NRM in a multivariate analysis (hazard ratio [HR], 4.79; $P < .001$; supplemental Table 4). As expected, the greatest contributor to NRM after flares was acute GVHD or complications from its treatment (54/71 [76%]). The time to flare did not affect NRM; patients with early flares (defined as occurring <28 days from CR/VGPR) experienced the same 6-month NRM as patients with late flares (27% [95% CI, 19.1-35.9] vs 27% [95% CI, 18.4-35.5]; $P = .962$). Similarly, flares during steroid tapers (151/210 [72%]) and after steroid discontinuation (59/210 [28%]) had similar 6-month NRM from the onset of flares (26% [95% CI, 19.3-33.3] vs 29% [95% CI, 18.0-41.0]; $P = .638$).

Consistent with the unexpectedly high risk of NRM, the clinical severity of acute GVHD at the onset of flare was greater than that of the original episode (grade 3-4, 41% vs 16%; $P = .001$; Figure 2B; supplemental Table 5). This increased severity was mainly because of greater lower gastrointestinal (LGI) involvement (55% vs 32%; $P < .001$; Figure 2C; supplemental Table 5). Grade 3/4 GVHD and/or LGI involvement were not more frequent at onset of GVHD among patients who had a subsequent flare than among those who did not.

Prediction of flares at CR/VGPR. The MAP combines the concentrations of 2 serum biomarkers into a single value that can be considered a "liquid biopsy" of crypt damage throughout the GI tract.[13,23] The MAP value determines the risk of 6-month NRM and is used to calculate the AA score that has been validated as a predictive biomarker by several research groups.[34,35] The MAP detects damage to GI crypts even before symptoms appear,[21] and we, therefore, measured MAPs in all patients at the time of CR/VGPR. The incidence of eventual flare increased with each increase in AA score (AA1, 16%; AA2, 26%; AA3, 39%; $P < .001$; Figure 3A), as did the severity of flare and the proportion of patients with LGI involvement (Figure 3B). In an exploratory analysis, we calculated the Akaike information criterion for predicting GVHD flares based on the AA score at treatment initiation (2510.3), at CR/VGPR (2492.5), and at both time points (2494.1). The lowest Akaike information criterion was at CR/VGPR, suggesting that the time point closest to flare was the strongest predictor. A multivariate analysis confirmed AA scores at CR/VGPR as significant risk factors for that development of GVHD flares (AA2 vs AA1: HR, 1.80 [$P = .001$]; AA3 vs AA1: HR, 3.13 [$P < .001$]; Table 2). The AA score also predicted 6-month NRM at the time of first CR/VGPR (AA1, 5%; AA2, 11%; AA3, 34%; $P < .001$; Figure 3C).

Surprisingly, flares occurred more frequently among patients with early achievement of CR/VGPR (within 14 days) than those who responded later (>14 days; 23% vs 14%; $P = .016$), which was confirmed by a multivariate analysis (HR, 1.84; $P = .004$; Table 2). Other than HLA-mismatched unrelated donors (HR, 1.74; $P = .049$; Table 2), no additional clinical variables were associated with the development of flare (Table 2; supplemental Table 6). We reasoned that time to achieve CR/VGPR might influence steroid exposure for 2 reasons. First, as expected, the cumulative doses of steroids before CR/VGPR were significantly higher in patients who were slower to respond to treatment (median cumulative dose of steroids in mg/kg MPE, 21.3 vs 9.34; $P < .001$). Second, the time to achievement of CR/VGPR might influence how steroids were tapered; for example, patients whose GVHD was more sensitive to steroid treatment (ie, early responders) might be tapered more rapidly. To explore this possibility, we estimated the rate of steroid dose reduction for each patient using the difference in 2 steroid doses: at first CR/VGPR and at 4 weeks later, flare, death, or discontinuation of steroids, whichever occurred first; we expressed this value as a weekly reduction rate. We observed an interaction between the taper rate and the maximum steroid dose for GVHD flares (supplemental Figure 2), and thus, we incorporated the maximum steroid dose in the determination of slow and rapid taper groups (supplemental Methods). The slow taper group included patients who were tapered <20% per week if the maximum steroid dose was ≤1 mg/kg MPE or <30% per week if the maximum steroid dose was >1 mg/kg MPE; all other patients were included in the rapid taper group. Patients whose steroids were tapered rapidly were nearly twice as likely to

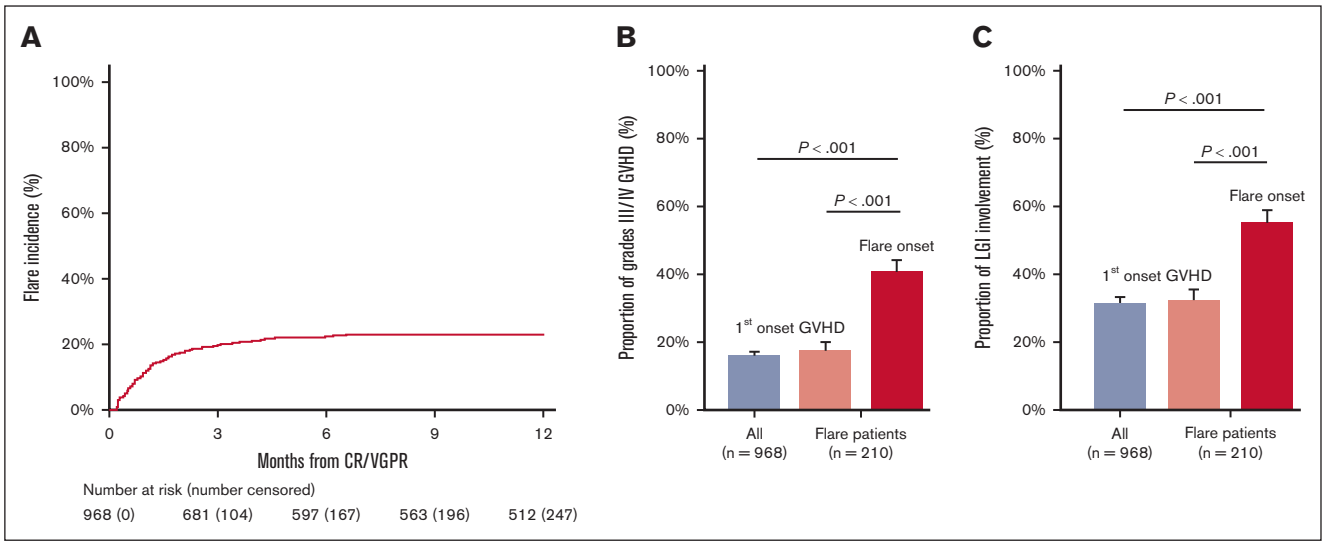

Figure 2. Incidence, severity, and LGI involvement of flares. (A) The cumulative incidence of flares after CR/VGPR; the cumulative incidence at 6 month was 21.6% (95% CI, 19.0-24.2). (B) The proportion of grades 3/4 acute GVHD at first onset in the entire population (n = 968, 16.3%), in patients with flare (n = 210, 18.1%), and at the time of flare (n = 210, 41.4%). (C) The proportion of LGI involvement at first onset in the entire population (n = 968, 31.6%), in patients with flare (n = 210, 32.9%), and at the time of flare (n = 210, 55.2%). *P* values for pairwise comparisons were adjusted using a Bonferroni method. The error bars represent standard error.

experience a flare compared with patients who were tapered slowly (30% vs 17%; $P < .001$; Figure 4A). Interestingly, patients who achieved CR/VGPR <14 days were just as likely to be tapered slowly as patients who took longer to achieve CR/VGPR (504/796 [63%] vs 97/172 [56%]; $P = .100$). A multivariate analysis revealed that the rate of steroid taper (HR, 2.65; $P < .001$), AA scores, and time to CR/VGPR were all significant and independent risk factor of flares (Table 2). We further explored the relationship among these risk factors in a stepwise fashion. First, graphical visualization of the 2 strongest risk factors, AA score and steroid taper, showed that patients tapered rapidly were more likely to flare than patients tapered slowly, and the difference among taper groups was larger for patients with AA2/3 GVHD (Figure 4B-C). The interaction between AA score at CR/VGPR and steroid taper was highly significant ($P = .008$), that is, the benefit of slow tapers was greater for patients with AA2/3 GVHD (supplemental Table 7). Finally, we examined the relationship among all 3 risk factors (supplemental Table 8). In this

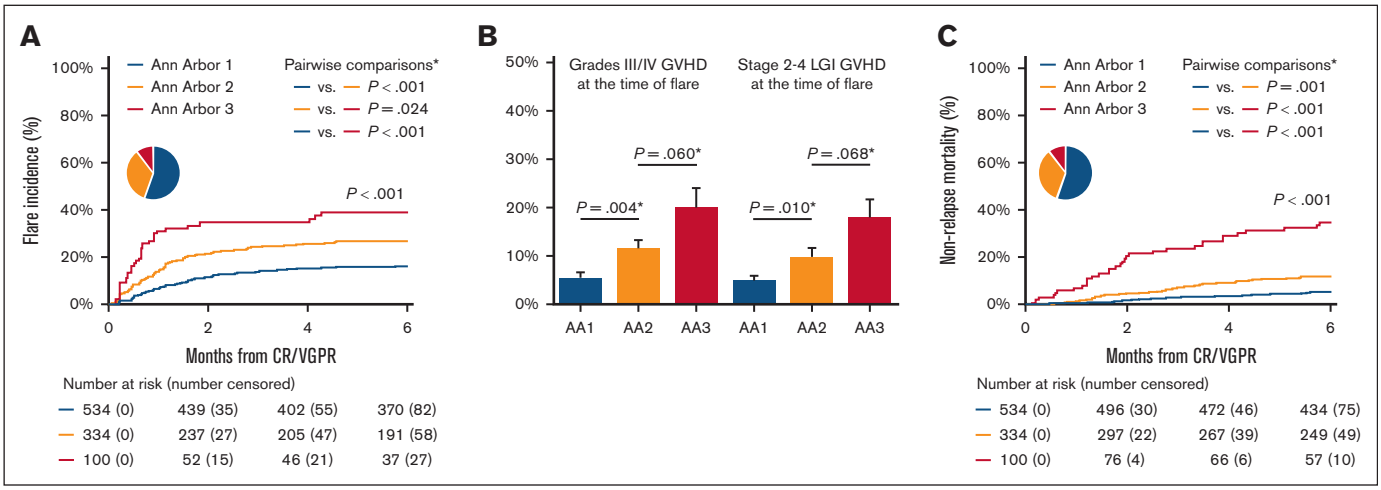

Figure 3. Association of biomarkers at CR/VGPR on GVHD flare, its severity, and NRM. (A) The cumulative incidence of flares after CR/VGPR stratified by the AA score at CR/VGPR; the cumulative incidence at 6 month was 15.8% (95% CI, 12.8-19.0) in AA1, 26.1% (95% CI, 21.4-30.9) in AA2, and 38.6% (95% CI, 28.6-48.5) in AA3. (B) The proportion of patients who developed grades 3/4 GVHD flare stratified by the AA scores at CR/VGPR (left panel); 5.4% (29/534) in AA1, 11.4% (38/334) in AA2, and 20.0% (20/100) in AA3. The proportion of patients who developed GVHD flare with stage 2-4 LGI involvement stratified by the AA scores at CR/VGPR (right panel); 4.9% (26/534) in AA1, 9.9% (33/334) in AA2, and 18.0% (18/100) in AA3. The error bars represent standard error. (C) The cumulative incidence of NRM after CR/VGPR stratified by MAP biomarkers at CR/VGPR; the cumulative incidence at 6 months was 4.7% (95% CI, 3.2-6.8) in AA1, 11.0% (95% CI, 7.9-14.7) in AA2, and 33.5% (95% CI, 24.4-43.3) in AA3. The pie chart shows the percentage of AA1 (blue), AA2 (yellow), and AA3 (red). **P* values for pairwise comparisons were adjusted using a Bonferroni method.

Table 2. Risk factors of flares within 6 months after CR/VGPR

	Without steroid taper rates		With steroid taper rates	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Biomarker score at CR/VGPR				
AA1	1	Ref	1	Ref
AA2	1.80 (1.31-2.45)	<.001	1.80 (1.31-2.47)	<.001
AA3	3.13 (1.97-4.96)	<.001	3.17 (1.99-5.06)	<.001
Recipient age, y				
18-54	1	Ref	1	Ref
<18	1.22 (0.72-2.06)	.460	0.99 (0.57-1.71)	.970
≥55	1.04 (0.75-1.44)	.820	0.99 (0.71-1.37)	.950
Sex mismatch				
Others	1	Ref	1	Ref
Female to male	1.45 (0.99-2.13)	.055	1.43 (0.97-2.11)	.067
Donor type				
HLA-matched related	1	Ref	1	Ref
HLA-matched unrelated	1.16 (0.76-1.78)	.490	1.23 (0.79-1.90)	.360
HLA-mismatched unrelated	1.74 (1.00-3.02)	.049	1.72 (0.98-3.03)	.060
Haploidentical	1.14 (0.52-2.48)	.740	1.36 (0.61-3.03)	.450
Umbilical cord blood	1.50 (0.69-3.26)	.300	1.89 (0.88-4.06)	.100
GVHD prophylaxis				
CNI based	1	Ref	1	Ref
PTCy based	1.01 (0.56-1.81)	.990	0.92 (0.50-1.70)	.800
Others	1.30 (0.64-2.61)	.470	1.25 (0.63-2.49)	.520
HCT-CI				
0-2	1	Ref	1	Ref
≥3	1.15 (0.85-1.56)	.370	1.19 (0.87-1.62)	.280
Use of ATG or alemtuzumab				
No	1	Ref	1	Ref
Yes	0.95 (0.70-1.30)	.760	0.81 (0.58-1.12)	.200
Conditioning				
MAC (TBI <8 Gy)	1	Ref	1	Ref
MAC (TBI ≥8 Gy)	0.76 (0.49-1.18)	.220	0.73 (0.46-1.15)	.180
RIC	1.07 (0.77-1.48)	.680	0.98 (0.71-1.34)	.870
Max grades before CR/VGPR				
Grades 1-2	1	Ref	1	Ref
Grades 3-4	1.02 (0.69-1.49)	.930	1.00 (0.68-1.45)	.990
Timing of achievement of CR/VGPR				
Late response (>14 d)	1	Ref	1	Ref
Early response (≤14 d)	1.80 (1.17-2.77)	.007	2.15 (1.40-3.30)	<.001
Max steroids dose before CR/VGPR*				
≥1 mg/kg	1	Ref	1	Ref
<1 mg/kg	1.07 (0.78-1.48)	.670	1.40 (0.99-1.98)	.053
GVHD treatment before CR/VGPR				
Steroid alone	1	Ref	1	Ref
Steroid + ruxolitinib	0.77 (0.22-2.68)	.680	0.98 (0.28-3.44)	.970
Steroid + other†	1.22 (0.77-1.92)	.400	1.44 (0.91-2.27)	.120
Treatment response				

ATG, antithymocyte globulin; CNI, calcineurin inhibitor; MAC, myeloablative conditioning; Ref, reference; RIC, reduced intensity conditioning; TBI, total body irradiation.
*Dose of methylprednisolone or equivalent.
†Excludes ruxolitinib.

Table 2 (continued)

	Without steroid taper rates		With steroid taper rates	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
CR	1	Ref	1	Ref
VGPR	1.11 (0.81-1.52)	.510	1.21 (0.88-1.66)	.250
Steroid taper rates				
Slow tapers			1	Ref
Rapid tapers			2.65 (1.93-3.64)	<.001

ATG, antithymocyte globulin; CNI, calcineurin inhibitor; MAC, myeloablative conditioning; Ref, reference; RIC, reduced intensity conditioning; TBI, total body irradiation.
*Dose of methylprednisolone or equivalent.
†Excludes ruxolitinib.

analysis, an early CR/VGPR was associated with higher incidence of flares in patients with AA1 GVHD at the time of response whether they were tapered slowly or rapidly, but the effect was not observed for patients with AA2/3 GVHD at the time of response.

Biomarkers at the onset of flare. Biomarker scores predict NRM at several time points other than GVHD onset,[13,22,24] and we, therefore, assessed their predictive value at the time of flare. Serum samples were available in 98 of 210 patients (47%) at the time of flare. The difference in 6-month cumulative incidence of NRM was not statistically significant for patients with and without samples (22% vs 32%; $P = .108$). The 6-month incidence of NRM from the time of flare steadily increased with each increase in AA score (AA1, 6%; AA2, 19%; AA3, 42%; $P = .001$; supplemental Figure 3).

Discussion

Flares of acute GVHD are often observed during tapering or after discontinuation of immunosuppressive therapy, however their significance with respect to overall outcomes is not well understood. Although flares are recognized as important in clinical GVHD trials either as an end point (ie, duration of response)[36,37] or as an eligibility criterion for steroid-refractory/dependent GVHD,[9,38-40] there is no established definition. Relying on expert consensus in our consortium, in this study, we define a flare of acute GVHD as worsening of symptoms by at least 1 stage in at least 1 organ after an initial CR/VGPR that prompted an increase or restart of immunosuppressive treatment.

In this large multicenter study that used granular clinical data of weekly GVHD grading and treatments uniformly collected by the MAGIC, the incidence of flares was 22% at 6-months from initial treatment response. Flares were more severe than the initial episode of acute GVHD, mainly due to more LGI involvement, which resulted in a significantly higher risk of NRM. At the time of CR/VGPR, elevated MAP predicted the future occurrence of a flare and its severity. At flare onset, MAGIC biomarkers also successfully stratify patients for the risk of NRM similarly to their performance in de novo acute GVHD.[23-25,41] These findings are consistent with prior studies showing that biomarkers can predict outcomes at specified time points after initiation of

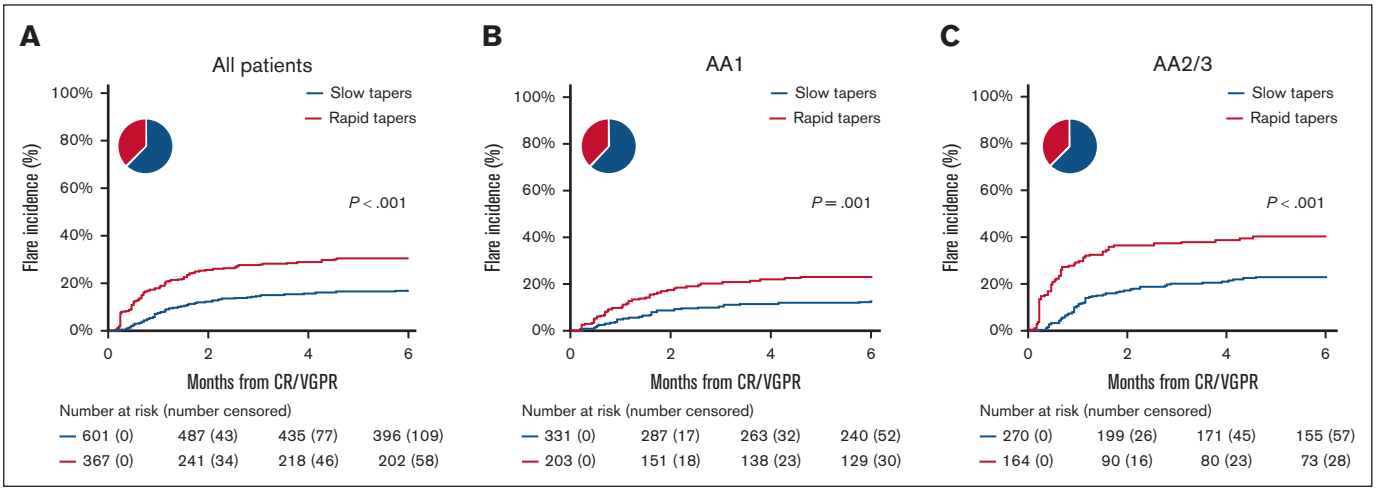

Figure 4. Associations of steroid tapers on flares in all patients and patients with AA1 and AA2/3. (A) The cumulative incidence of flares was 16.5% (95% CI, 13.6-19.6) in slow tapers and 30.0% (95% CI, 25.3-34.8) in rapid tapers. (B) Only patients with AA1 at CR/VGPR. The cumulative incidence of flares was 11.9% (95% CI, 8.7-15.7) in slow tapers and 22.1% (95% CI, 16.6-28.1) in rapid tapers. (C) Only patients with AA2/3 at CR/VGPR. The cumulative incidence of flares was 22.3% (95% CI, 17.4-27.5) in slow tapers and 39.8% (95% CI, 32.1-37.4) in rapid tapers. Tapers were defined as rapid if the weekly steroid reduction rate ≥ 30% per week when the maximum steroid dose before CR/VGPR ≥1 mg/kg or ≥ 20% per week when the maximum steroid dose before CR/VGPR <1 mg/kg. The pie chart shows the percentage of patients whose steroid taper was slow (blue) and rapid (red).

treatment (eg, day 28)[13] and emphasize that the MAP reflects subclinical damage to the GI crypts when clinical symptoms are well controlled.

In addition to an increase in serum MAP scores, 3 clinical risk factors for GVHD flare were identified. One was the use of an HLA-mismatched unrelated donor, a well-known factor of severe acute GVHD.[28] Given the lack of significant difference in posttransplant cyclophosphamide (PTCy) haploidentical donor HCT in our analysis, GVHD prophylaxis with PTCy might overcome the risk of flares in HLA-mismatched settings, but we could not perform subgroup analysis stratified by GVHD prophylaxis because of limited sample size of nonhaploidentical HCT with PTCy. The 2 other clinical risk factors, earlier achievement of clinical response and a faster steroid taper, may be surrogates for cumulative steroid exposure before flare. These results should be interpreted cautiously because we calculated the weekly steroid reduction rate from limited data over a short time period. Our estimate assumed that the rate of steroid tapering was constant in the first month after CR/VGPR, when, in fact, physicians often adjust the rate when the initial steroid dose is higher[42] and continue to modify the rate as the dose of steroids decreases.[3] Nevertheless, our findings suggest that inadequate exposure to corticosteroids might contribute for GVHD flare, a possibility supported by the observation that the risk of flares increases when a rapid taper is combined with greater subclinical disease (ie, high MAP score). Taken together, our data suggest that the risk of flares may be modifiable using biomarkers to stratify the steroid tapering schedule. We recently initiated a prospective trial to test aggressive steroid tapers in responding patients guided by serial monitoring of biomarker parameters (registered at www.ClinicalTrials.gov as #NCT05090384). Notably, other clinical characteristics including higher peak GVHD severity, higher peak daily dose of steroids, or the use of additional immunosuppressive agents in combination with steroids were not significantly associated with increased risk of GVHD flare.

Our study has several limitations. First, we did not empirically derive our definition of flare, and it is possible that other definitions of flare may associate more strongly with NRM. Second, the treatment of these patients with GVHD was at the physician's discretion and reflected wide variation in practice. Patients who experienced a mild symptom increase (eg, skin stage 1 to 2) and who received an increase in immunosuppression were included, whereas patients with the same increase in GVHD symptoms who were not treated with increased immunosuppression were excluded because they did not meet the definition of flare. Third, patients who did not respond to primary treatment for GVHD within 28 days, who are considered poor prognostic populations,[5,14,43-45] were also excluded because our data/sample collection protocol is focused on the first 28 days from the onset of GVHD, with very limited samples available beyond 28 days. Fourth, treatment decisions regarding steroid dose and duration may be influenced by factors other than GVHD such as the presence of minimal residual disease,[46-49] concurrent infections,[50-52] and inflammatory conditions such as transplant-associated thrombotic microangiopathy[53-55] that are not consistently included in the database and, therefore, could not be considered in these analyses. Fifth, treatment decisions varied among physicians and transplant centers and reflect real world clinical practice. In our cohort, treatment for grade 1 acute GVHD was common, and there was wide variation in initial steroid dose, as has been observed elsewhere.[42,56,57] However, neither GVHD severity nor steroid dose before CR/VGPR correlated with the incidence of flares, underscoring the importance of careful management after clinical responses even in such cases. Finally, we were not able to model the kinetics of MAP over time in this study with limited data/samples available, although such dynamic and mathematical modeling may improve the prediction of GVHD flare in the future.

In conclusion, this study described incidence, risk factors, and outcomes of GVHD flare after initial clinical response. GVHD flares among patients who achieved CR/VGPR are common and result in higher NRM. This study emphasizes the significance of GVHD flares in clinical practice, and our consensus definition of flares enables future clinical studies to accurately report the incidence and impact of experimental treatments on flares. MAGIC biomarkers, which can detect residual acute GVHD activity and damage, help quantify the risk for flares and their severity and may guide future treatment strategies including steroid tapers.

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Authorship

Contribution: Y.A. designed the study, collected the clinical data, conducted the statistical analysis, and wrote the manuscript; N.S. collected the clinical data, advised statistical methods, and reviewed and revised the manuscript; M.H., P.A.-H., F.A., C.C., H.K.C., M.E., A.M.E., S.A.G., E.O.H., W.J.H., C.L.K., S. Kraus, M.M.A.M., P.M., M.Q., R.R., T.S., E.U., I.V., M.W., R.Z., and Z.D. collected the clinical data, and reviewed and revised the manuscript; J.B., G.E., S.G., N.K., and R.Y. collected and reviewed the clinical data; S. Kasikis, R.B., S. Kowalyk, and G.M. performed the laboratory analysis; J.E.L., J.L.M.F., and R.N. interpreted data, advised methods, reviewed and revised the manuscript, and organized this project; and all authors contributed to the writing of the report and approved the final version of the article.

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ORCID profiles: Y.A., 0000-0001-6825-9340; P.A.-H., 0000-0002-0196-806X; S.A.G., 0000-0001-8030-7595; E.O.H., 0000-0002-1125-4060; W.J.H., 0000-0002-5841-4105; P.M., 0000-0001-6426-4046; M.Q., 0000-0001-7689-343X; R.R., 0000-

0003-2185-9546; E.U., 0000-0001-8530-1192; N.K., 0009-0004-7955-1119; Z.D., 0000-0002-7994-8974; J.E.L., 0000-0002-5611-7828; R.N., 0000-0002-9082-0680.

Correspondence: John E. Levine, The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Pl, Box 1410, New York, NY 10029; email: john.levine@mssm.edu.

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