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blood advances

Incidence, clinical presentation, risk factors, outcomes, and biomarkers in de novo late acute GVHD

Yu Akahoshi,[1,2] Nikolaos Spyrou,[1] William J. Hogan,[3] Francis Ayuk,[4] Zachariah DeFilipp,[5] Daniela Weber,[6] Hannah K. Choe,[7] Elizabeth O. Hexner,[8] Wolf Rösler,[9] Aaron M. Etra,[1] Karamjeet Sandhu,[10] Gregory A. Yanik,[11] Chantiya Chanswangphuwana,[12] Carrie L. Kitko,[13] Ran Reshef,[14] Sabrina Kraus,[15] Matthias Wölfl,[16] Matthias Eder,[17] Hannah Bertrand,[18] Muna Qayed,[19] Pietro Merli,[20] Stephan A. Grupp,[21] Paibel Aguayo-Hiraldo,[22] Tal Schechter,[23] Evelyn Ullrich,[24] Janna Baez,[1] Rahnuma Beheshti,[1] Sigrun Gleich,[6] Steven Kowalyk,[1] George Morales,[1] Rachel Young,[1] Deukwoo Kwon,[25] Ryotaro Nakamura,[10] John E. Levine,[1,*] James L. M. Ferrara,[1,*] and Yi-Bin Chen[5,*]

[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]Division of Hematology, Mayo Clinic, Rochester, MN; [4]Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [5]Hematopoietic Cell Transplant and Cellular Therapy Program, Massachusetts General Hospital, Boston, MA; [6]Department of Hematology and Oncology, Internal Medicine III, University of Regensburg, Regensburg, Germany; [7]Blood and Marrow Transplantation Program, The Ohio State University, Columbus, OH; [8]Department of Medicine, Division of Hematology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [9]Department of Internal Medicine 5, Hematology and Oncology, Friedrich-Alexander-Universität Erlangen-Nürnberg and University Hospital Erlangen, Erlangen, Germany; [10]Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, Duarte, CA; [11]Blood and Marrow Transplantation Program, University of Michigan, Ann Arbor, MI; [12]Department of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand; [13]Pediatric Stem Cell Transplant Program, Vanderbilt University Medical Center, Nashville, TN; [14]Blood and Marrow Transplantation Program and Columbia Center for Translational Immunology, Columbia University Medical Center, New York, NY; [15]Department of Internal Medicine II and [16]Pediatric Blood and Marrow Transplantation Program, Children's Hospital, University Hospital of Würzburg, Würzburg, Germany; [17]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany; [18]Department of Medicine I, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [19]Emory University School of Medicine, Atlanta, GA; [20]Department of Haematology-Oncology and Cell and Gene Therapy, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [21]Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [22]Cancer and Blood Disease Institute, Children's Hospital Los Angeles, Los Angeles, CA; [23]Division of Hematology/Oncology/BMT, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; [24]Frankfurt Cancer Institute, Goethe University Frankfurt, Frankfurt am Main, Germany; and [25]Department of Population Health Science and Policy, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY

Key Points

- Compared with classic acute GVHD, late acute GVHD presents with worse clinical severity and higher-risk biomarker parameters.
- Patients with late acute GVHD have equivalent long-term survival outcomes compared with patients with classic acute GVHD.

Late acute graft-versus-host disease (GVHD) is defined as de novo acute GVHD presenting beyond 100 days after allogeneic hematopoietic cell transplantation (HCT) without manifestations of chronic GVHD. Data are limited regarding its characteristics, clinical course, and risk factors because of underrecognition and changes in classification. We evaluated 3542 consecutive adult recipients of first HCTs at 24 Mount Sinai Acute GVHD International Consortium (MAGIC) centers between January 2014 and August 2021 to better describe the clinical evolution and outcomes of late acute GVHD. The cumulative incidence of classic acute GVHD that required systemic treatment was 35.2%, and an additional 5.7% of patients required treatment for late acute GVHD. At the onset of symptoms, late acute GVHD was more severe than classic acute GVHD based on both clinical and MAGIC algorithm probability biomarker parameters and showed a lower overall response rate on day 28. Both clinical and biomarker grading at the time of treatment stratified the risk of nonrelapse mortality (NRM) in patients with classic and late acute GVHD, respectively, but long-term NRM and overall survival did not differ between patients with classic and late acute GVHD. Advanced age, female-to-male sex mismatch, and the use of reduced intensity conditioning were associated with the development of late acute GVHD, whereas the use of posttransplant cyclophosphamide–based GVHD prevention was protective mainly because of

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*J.E.L., J.L.M.F., and Y.-B.C. contributed equally to this study.

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

shifts in GVHD timing. Because overall outcomes were comparable, our findings, although not definitive, suggest that similar treatment strategies, including eligibility for clinical trials, based solely on clinical presentation at onset are appropriate.

Introduction

Acute graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT).[1] For decades, any clinical manifestations of GVHD before day 100 were defined as acute GVHD, and any GVHD symptoms after day 100 were considered chronic GVHD.[2] The 2005 National Institutes of Health (NIH) Consensus Conference proposed new diagnostic criteria for GVHD based purely on clinical manifestations without any reference to the time of onset.[3] Acute GVHD was classified as classic if it developed before day 100, late if it developed after day 100 without prior acute GVHD, recurrent if it recurred after day 100 following a previous resolution of symptoms, and persistent if symptoms continued after day 100 without resolution.

Following the publication of the NIH criteria, several studies showed that patients who developed acute GVHD after day 100 experienced higher nonrelapse mortality (NRM) than patients who did not develop acute GVHD,[4-7] and these patients often had outcomes equivalent to those of patients with chronic GVHD.[8-11] However, acute GVHD after day 100 described in these studies encompassed all subtypes, including late, recurrent, and persistent. There is conflicting evidence for the prognostic implications of late acute GVHD,[5-8,10,12-14] but the relatively small sample size of these studies has made it difficult to draw definitive conclusions regarding the prognostic implications of late acute GVHD. In this retrospective analysis, we report on cases of new onset late acute GVHD derived from the Mount Sinai Acute GVHD International Consortium (MAGIC) and describe its incidence, clinical presentation, risk factors, outcomes, and value of GVHD biomarkers in this setting.

Methods

Study design and patient selection

MAGIC prospectively collects data from 24 HCT centers in North America, Europe, and Asia regarding the natural history of GVHD using a rigorous prospective randomized open, blinded end point study design.[15-17] Informed consent from an institutional review board–approved protocol was obtained from all participants in accordance with the Declaration of Helsinki. Patients were included in this analysis if they were aged ≥18 years, received their first HCT from an HLA-matched related donor (MRD), HLA-matched unrelated donor (MUD), HLA-mismatched unrelated donor (MMUD), or haploidentical donor between 1 January 2014 and 31 August 2021. Recipients who underwent HCT using umbilical cord blood or ex vivo T-cell depletion were excluded because of limited numbers of such patients.

Definitions

Acute GVHD was diagnosed and staged per the standard MAGIC consensus criteria.[17] Patients were prospectively monitored for acute GVHD per the institutional frequency until day 180, and data were collected in near real-time. GVHD that occurred after day 180 was identified via retrospective chart review. Acute GVHD that first developed before day 100 was defined as classic, whereas acute GVHD that first developed after day 100 was defined as late acute GVHD. Transaminase elevation without hyperbilirubinemia was not diagnosed as acute GVHD per MAGIC criteria, unlike certain other studies.[4,5,18] Chronic GVHD was diagnosed per NIH consensus criteria.[19] Patients who developed acute GVHD after disease relapse or donor lymphocyte infusion, or those who possessed clinical manifestations that overlapped with chronic GVHD at the time of initiation of systemic treatment were excluded.

We defined systemic treatment for acute GVHD as a minimum of 10 mg methylprednisolone, or equivalent, per day. Complete response (CR) was defined as the complete resolution of acute GVHD manifestations within 28 days of treatment initiation without any additional treatment. Partial response (PR) was defined as improvement in at least 1 organ without achieving CR or worsening in any other organ and not requiring any additional systemic treatment beyond corticosteroids before day 28. HCT-specific comorbidity index scores, conditioning regimen intensity, and Minnesota risk were classified as previously reported.[20-22] Primary diseases were classified as high risk for the following: acute myeloid leukemia or lymphoma after induction failure, active relapse (including stable or progressive disease for lymphoma or chronic lymphocytic leukemia, excluding indolent lymphoma), refractory anemia with excess blasts, Burkitt lymphoma, acute lymphoblastic leukemia in second remission or greater, or chronic myeloid leukemia in blast phase. All other hematological disorders were categorized as standard risk.

Sample collection

Serial serum samples were collected prospectively and cryopreserved at the time of systemic treatment for acute GVHD and after 1 week of treatment for patients enrolled on the MAGIC natural history biorepository study. Serum levels of suppressor of tumorigenicity-2 (ST2)[23] and regenerating islet-derived protein 3-α (Reg3α)[24] were measured retrospectively, using enzyme-linked immunosorbent assays, as previously described.[25-27] The MAGIC algorithm probability (MAP) was calculated as a single value between 0.001 and 0.999 per this formula: $\log[-\log(1 - \text{MAP})] = -11.263 + 1.844(\log_{10}\text{–ST2}) + 0.577(\log_{10}\text{–Reg3}\alpha)$.[25,26,28] Validated MAP thresholds were used for stratification at the initiation of systemic treatment (Ann Arbor [AA] 1 < 0.14; 0.14 ≤ AA 2 < 0.29; AA 3 ≥ 0.29)[25] and after 1 week of treatment (low, <0.29; high, ≥0.29).[26]

Statistical analysis

Categorical variables of groups of patients were compared using the Fisher exact test. Continuous variables were compared using the Mann-Whitney *U* test. Competing risks for the cumulative incidence of acute GVHD were relapse or death without acute

GVHD; the competing risk for NRM was relapse; and the competing risk for relapse was death without relapse. Differences in cumulative incidences between groups were calculated using the Gray test. The Fine and Gray method was used to evaluate the risk factors for acute GVHD. The association of acute GVHD as a time-dependent covariate was evaluated using a cause-specific Cox proportional hazards regression model.[29] Only patients without a prior history of classic acute GVHD who survived without relapse till day 100 were included in analyses of late acute GVHD. When assessing the associations with clinical and biomarker parameters on outcomes and analyzing only patients with GVHD, the time to acute GVHD onset was incorporated as a binary covariate (classic vs late) in models, and all outcomes were censored at 6 months after starting systemic treatment. We compared survival outcomes between the 2 groups using the log-rank test, Gray model, Fine and Gray model, or Cox proportional hazards regression model, as appropriate. The cumulative incidence of overall acute GVHD with systemic treatment at 3 months and 12 months after HCT were compared to identify covariates significantly associated with late acute GVHD.[30]

The following potential covariates were included in multivariate analyses: recipient age at HCT (<55 vs ≥55 years), sex mismatch (female donor–to–male recipient vs other), primary disease (acute leukemia vs myelodysplastic syndromes, or myeloproliferative neoplasms vs malignant lymphoma vs others), disease risk (standard vs high), donor type (MRD vs MUD vs MMUD vs haploidentical donor), GVHD prophylaxis (methotrexate and calcineurin inhibitor [CNI]-based vs mycophenolate mofetil and CNI-based vs posttransplant cyclophosphamide [PTCy]-based vs others), HCT-specific comorbidity index scores (<3 vs ≥3), use of in vivo T-cell depletion (no vs yes), donor source (bone marrow vs peripheral blood), and conditioning regimen intensity (total body irradiation–based myeloablative conditioning [MAC] vs non–total body

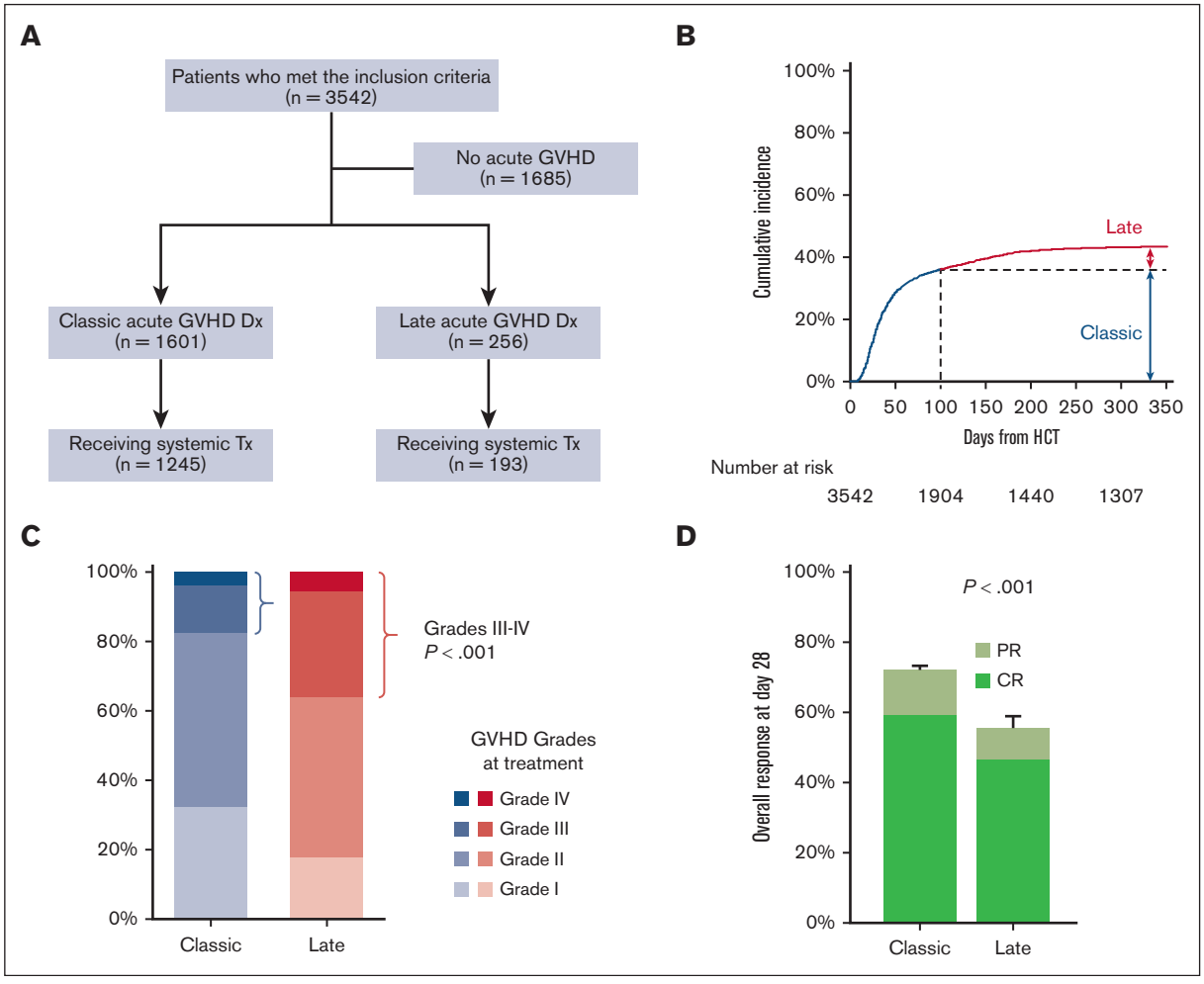

Figure 1. Incidence, overall grades, and overall response of acute GVHD that required treatment. (A) Among the 3542 patients who met the criteria for inclusion, 1601 and 256 were diagnosed with classic and late acute GVHD of any grade, respectively. Of patients with classic and late acute GVHD, 1245 of 1601 (77.8%) and 193 of 256 (75.4%), respectively, received systemic GVHD treatment. (B) The cumulative incidence of classic and late acute GVHD that required systemic treatment were 35.2% (95% CI, 33.6-36.8) and 5.7% (95% CI, 4.9-6.5), respectively. (C) The rates of grades 3/4 acute GVHD were 17.7% in classic and 36.3% in late acute GVHD ($P < .001$). (D) Overall response rate (CR or PR) on day 28 was 72.0% in classic vs 55.4% in late acute GVHD ($P < .001$). CR rate on day 28 was 59.3% in classic and 46.6% in late acute GVHD ($P < .001$). Dx, diagnosis; CR, complete response; PR, partial response; Tx, treatment.

Table 1. Patient characteristics with systemic treatment

	Classic n = 1245	Late n = 193	*P* value
Median age at HCT, y (range)	58 (18-79)	61 (19-78)	< .001
Recipient age, category			
<55	392 (31.5)	33 (17.1)	< .001
≥55	853 (68.5)	160 (82.9)	
Sex mismatch			
Other	1057 (84.9)	145 (75.1)	.001
Female-to-male	188 (15.1)	48 (24.9)	
Primary disease			
Acute leukemia	653 (52.4)	100 (51.8)	.475
MDS/MPN	349 (28.0)	63 (32.6)	
Malignant lymphoma	103 (8.3)	12 (6.2)	
Other	140 (11.2)	18 (9.3)	
Disease risk			
Standard	991 (79.6)	165 (85.5)	.064
High	254 (20.4)	28 (14.5)	
Donor type			
HLA-MRD	256 (20.6)	86 (44.6)	<.001
HLA-MUD	708 (56.9)	88 (45.6)	
HLA-MMUD	131 (10.5)	8 (4.1)	
Haploidentical donor	150 (12.0)	11 (5.7)	
GVHD prophylaxis			
CNI/MTX based	649 (52.1)	135 (69.9)	<.001
CNI/MMF based	290 (23.3)	37 (19.2)	
PTCy	248 (19.9)	13 (6.7)	
Other	58 (4.7)	8 (4.1)	
HCT-CI			
0-2	814 (65.4)	120 (62.2)	.418
≥3	431 (34.6)	73 (37.8)	
In vivo T-cell depletion			
No	760 (61.0)	149 (77.2)	<.001
Yes	485 (39.0)	44 (22.8)	
Donor source			
Bone marrow	211 (16.9)	19 (9.8)	.011
Peripheral blood	1034 (83.1)	174 (90.2)	
Conditioning			
MAC (TBI < 8 Gy)	516 (41.4)	51 (26.4)	<.001
MAC (TBI ≥ 8 Gy)	179 (14.4)	15 (7.8)	
RIC	550 (44.2)	127 (65.8)	
CNI discontinuation by day 100			
Discontinued		13 (6.7)	NA
Continued		180 (93.3)	
Median year of HCT (range)	2017 (2014-2021)	2018 (2014-2021)	.120

HCT-CI, HCT-specific comorbidity index; MDS/MPN, myelodysplastic syndromes/myeloproliferative neoplasms; MMF, mycophenolate mofetil; MTX, methotrexate; TBI, total body irradiation.

Table 2. GVHD characteristics at treatment

	Classic n = 1245	Late n = 193	*P* value
GVHD grades II-IV			
1	401 (32.2)	34 (17.6)	< .001
2-4	844 (67.8)	159 (82.4)	
GVHD grades III-IV			
1-2	1025 (82.3)	123 (63.7)	< .001
3-4	220 (17.7)	70 (36.3)	
Minnesota risk			
Standard	1058 (85.0)	135 (69.9)	< .001
High	187 (15.0)	58 (30.1)	
Skin stage II-IV			
0-1	656 (52.7)	107 (55.4)	.486
2-4	589 (47.3)	86 (44.6)	
Lower gastrointestinal stage II-IV			
0-1	1056 (84.8)	130 (67.4)	< .001
2-4	189 (15.2)	63 (32.6)	
Liver gastrointestinal stage II-IV			
0-1	1211 (97.3)	183 (94.8)	.073
2-4	34 (2.7)	10 (5.2)	
Upper gastrointestinal stage			
0	868 (69.7)	124 (64.2)	.133
1	377 (30.3)	69 (35.8)	
Initial corticosteroid dose (median methylprednisolone [mg/kg], range)	0.91 (0.09-10.72)	0.83 (0.16-2.37)	.713
Use of other agents in addition to systemic steroids	102 (8.2)	15 (7.8)	1
Median days of initial treatment (range)	30 (5-99)	151 (102-330)	
GVHD grades			
1	401 (32.2)	34 (17.6)	< .001
2	624 (50.1)	89 (46.1)	
3	172 (13.8)	59 (30.6)	
4	48 (3.9)	11 (5.7)	
Skin stage			
0	407 (32.7)	85 (44.0)	< .001
1	249 (20.0)	22 (11.4)	
2	339 (27.2)	36 (18.7)	
3	241 (19.4)	50 (25.9)	
4	9 (0.7)	0 (0.0)	
Lower gastrointestinal stage			
0	842 (67.6)	98 (50.8)	< .001
1	214 (17.2)	32 (16.6)	
2	92 (7.4)	29 (15.0)	
3	62 (5.0)	23 (11.9)	
4	35 (2.8)	11 (5.7)	
Liver stage			
0	1195 (96.0)	178 (92.2)	.090
1	16 (1.3)	5 (2.6)	
2	20 (1.6)	5 (2.6)	
3	9 (0.7)	4 (2.1)	
4	5 (0.4)	1 (0.5)	

irradiation–based MAC vs reduced intensity conditioning [RIC]). In multivariate analyses of late acute GVHD and survival outcomes, CNI discontinuation by day 100 after HCT (discontinued vs continued) and year of HCT were also included.

Statistical significance was defined as a 2-tailed P value $< .05$. All statistical analyses were performed with EZR version 1.53 (Jichi Medical University Saitama Medical Center), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.2.2, Vienna, Austria).[31]

Results

Patient characteristics

Baseline characteristics of 3542 patients who received HCT from 2014 to 2021 and whose data were submitted to the MAGIC database are summarized in supplemental Table 1. The median age at HCT was 58 years (range, 18-79 years). In total, 81.2% of patients underwent HCT from MRD or MUD, 7.8% from MMUD, and 10.9% from haploidentical donors. The median follow-up time for survivors was 722 days after HCT. Among these, 1857 (52.4%) patients were diagnosed with acute GVHD of any grade, not all of whom required systemic treatment. Classic and late acute GVHD were identified in 1601 and 256 patients, respectively (Figure 1A; supplemental Table 2). Median days from HCT to onset of classic acute GVHD and late acute GVHD were day 30 (range, 5-99 days) and day 148 (range, 100-314 days), respectively.

The cumulative incidence of acute GVHD that required treatment was 40.9% (35.2% for classic acute GVHD; 5.7% for late acute GVHD; Figure 1B). Of the patients with classic and late acute GVHD, 1245 of 1601 (77.8%) and 193 of 256 (75.4%), respectively, received systemic GVHD treatment (Table 1). The median follow-up for survivors with classic and late acute GVHD was 679 days (range, 29-1726 days) and 546 days (range, 32-921 days) after HCT, respectively. Overall grades and, specifically, lower gastrointestinal organ staging were significantly higher in patients who presented with late acute GVHD compared with patients with classic acute GVHD (Figure 1C; Table 2). The initial dose of systemic corticosteroids prescribed did not significantly differ between classic and late acute GVHD groups (median dose, 0.91 vs 0.83 mg/kg per day methylprednisolone equivalent; $P = .713$; Table 2). Among the patients who received systemic treatment, 184 of 1245 (14.8%) of patients with classic acute GVHD and 44 of 193 (22.8%) of those with late acute GVHD received second-line acute GVHD treatment by day 28 after initiation of therapy ($P = .006$). The overall response rate (CR or PR) by day 28 was significantly higher in patients with classic acute GVHD compared with those with late acute GVHD (72.0% vs 55.4%; $P < .001$; Figure 1D).

Long-term outcomes

In multivariate analyses, development of either classic or late acute GVHD as a time-dependent covariate was both associated with an increased risk of NRM (classic: hazard ratio [HR], 2.53; 95% confidence interval [CI], 2.13-3.02; $P < .001$; and late: HR, 4.37; 95% CI, 3.03-6.30; $P < .001$) and inferior OS (classic: HR, 1.50; 95% CI, 1.32-1.70; $P < .001$; and late: HR, 2.29; 95% CI, 1.72-3.04; $P < .001$) (Table 3). Classic acute GVHD was associated with a decreased risk of disease relapse (HR, 0.77; 95% CI, 1.32-1.70; $P = .001$), whereas occurrence of late acute GVHD did not show any significant association with relapse (HR, 1.26; 95% CI, 0.86-1.86; $P = .236$). Although the clinical severity of acute GVHD at presentation was greater in late acute GVHD than in classic acute GVHD, there was no significant difference in rates of NRM at 6 months between the groups (at 6 months: 15.2% [95% CI, 13.3-17.3] vs 16.8% [95% CI, 11.8-22.6], $P = .551$; Figure 2A). Similar rates of underlying disease relapse (at 6 months: 11.6% [95% CI, 9.9-13.5] vs 11.9% [95% CI, 7.7-17.0]; $P = .869$) and OS (at 6 months: 79.5% [95% CI, 77.1-81.6] vs 78.2% [95% CI, 71.5-83.6]; $P = .827$) were also observed between the groups (Figure 2B-C). As expected, clinical severity (grades 1/2 vs 3/4) at treatment initiation stratified 6-month NRM in both classic (at 6 months: 11.9% [95% CI, 10.0-30.7] vs 30.7% [95% CI, 10.0-30.7]; $P < .001$; Figure 2D) and late acute GVHD (at 6 months: 8.4% [95% CI, 4.3-14.3] vs 32.5% [95% CI, 21.4-44.1]; $P < .001$; Figure 2E). In the multivariate analysis, clinical severity (HR, 3.23; 95% CI, 2.44-4.29; $P < .001$) was a significant risk factor for NRM, but the time to acute GVHD onset as either a binary covariate (HR, 0.72; 95% CI, 0.49-1.07; $P = .100$; Table 4) or continuous covariate (HR, 1.00; 95% CI, 0.99-1.00; $P = .730$) was not.

Table 3. Impact of GVHD on outcomes in the multivariate analyses

	HR (95% CI)	*P* value
NRM		
No classic	1	Ref
Classic	2.53 (2.13-3.02)	< .001
No classic/late	1	Ref
Late	4.37 (3.03-6.30)	< .001
Relapse		
No classic	1	Ref
Classic	0.77 (0.66-0.90)	.001
No classic/late	1	Ref
Late	1.26 (0.86-1.86)	.236
OS		
No classic	1	Ref
Classic	1.50 (1.32-1.70)	< .001
No classic/late	1	Ref
Late	2.29 (1.72-3.04)	< .001

GVHD was treated as a time-dependent covariate. All models were adjusted for recipient's age, sex mismatch, primary disease, disease risk, donor type, GVHD prophylaxis, HCT-CI, in vivo T-cell depletion, donor source, conditioning, and the year of HCT. In late acute GVHD analysis, CNI discontinuation by day 100 was also included. GVHD was treated as a time-dependent covariate. The number of events for NRM, relapse, and OS was 573, 806, and 1086, respectively.

Acute GVHD biomarkers

We evaluated the prognostic value of MAP biomarkers measured at the onset of acute GVHD. Serum samples were available at onset in 1041 of 1245 (83.6%) patients with classic acute GVHD and 89 of 193 (46.1%) patients with late acute GVHD (supplemental Table 3). There were no significant differences in the NRM between patients with and without samples (data not shown). More patients with late acute GVHD had high risk MAP biomarkers (AA3) than patients with classic acute GVHD (29.2% vs 16.2%; $P = .003$). As expected, patients with an AA1 score had a significantly lower risk of NRM than patients with AA2 or AA3 scores in

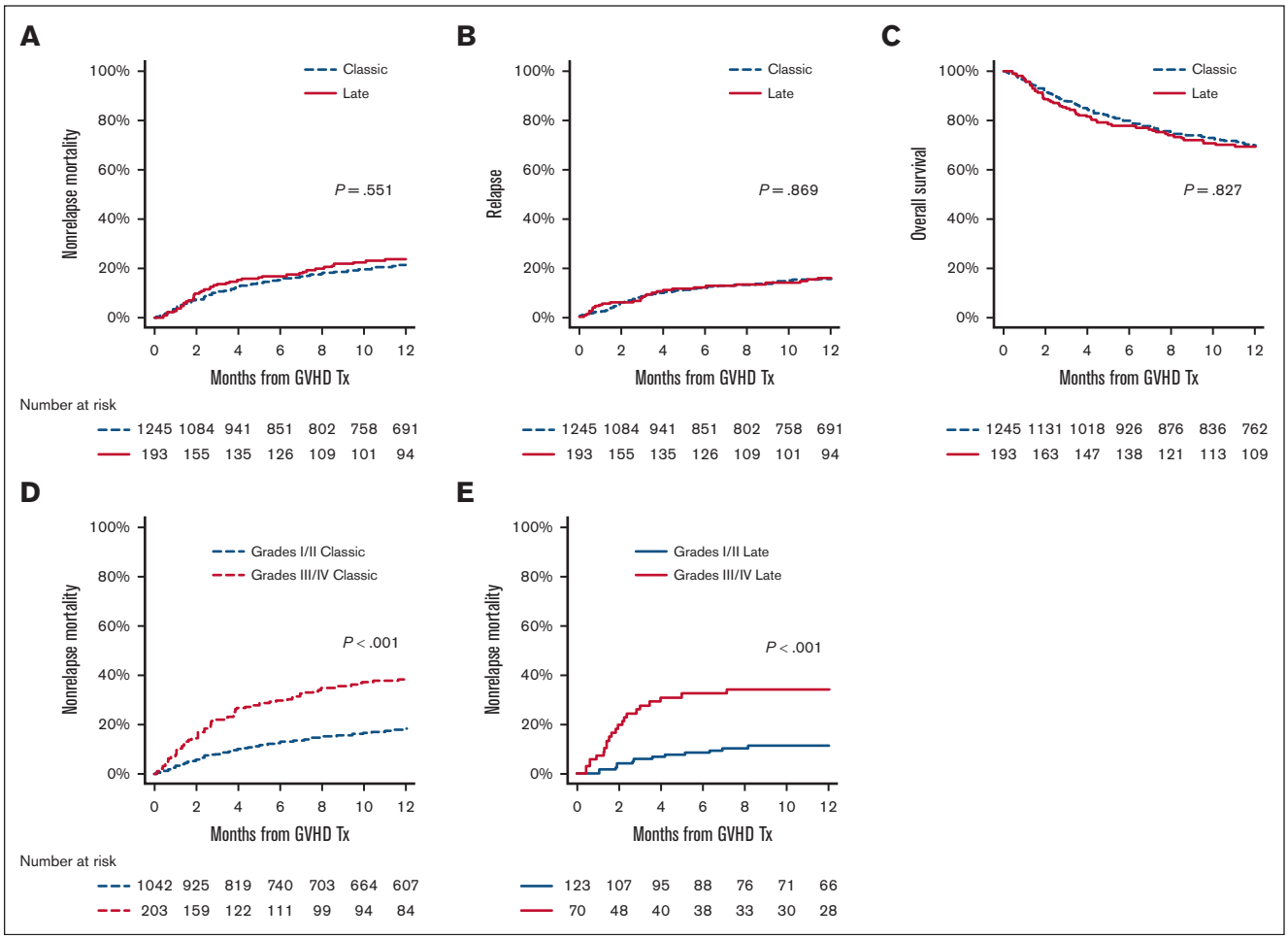

Figure 2. Long-term outcomes from the time of systemic treatment. (A) The cumulative incidences of NRM at 6 months were 15.2% (95% CI, 13.3-17.3) and 16.8% (95% CI, 11.8-22.6) in classic and late acute GVHD, respectively ($P = .551$). (B) The cumulative incidences of relapse at 6 months were 11.6% (95% CI, 9.9-13.5) and 11.9% (95% CI, 7.7-17.0) in classic and late acute GVHD, respectively ($P = .869$). (C) The probabilities of OS at 6 months were 79.5% (95% CI, 77.1-81.6) in classic and 78.2% (71.5-83.5) in late ($P = .827$). (D) The cumulative incidences of NRM at 6 months were 11.9% (95% CI, 10.0-14.0) and 30.7% (95% CI, 24.7-37.0) in grades 1/2 and grades 3/4 classic acute GVHD, respectively ($P < .001$). (E) The cumulative incidences of NRM at 6 months were 8.4% (95% CI, 4.3-14.3) and 32.5% (95% CI, 21.4-44.1) in grades 1/2 and grades 3/4 late acute GVHD, respectively ($P < .001$).

both classic (at 6 months: 7.4% [95% CI, 5.5-9.8] vs 25.9% [95% CI, 22.0-30.1]; $P < .001$) and late acute GVHD (at 6 months: 4.9% [95% CI, 0.9-14.7] vs 42.2% [95% CI, 27.1-56.6]; $P < .001$), respectively (Figure 3; supplemental Figure 1). In multivariate analysis, the biomarker score was a significant risk factor for NRM (HR, 3.52; 95% CI, 2.48-4.99; $P < .001$), but the time to acute GVHD onset as a binary covariate was not (HR, 0.87; 95% CI, 0.53-1.42; $P = .580$; Table 4).

We then evaluated the prognostic value of MAP biomarkers measured after initiating systemic treatment for acute GVHD because this could help clinical decisions for second-line treatment. Serum samples were available after 1 week of treatment in 687 of 1245 (55.2%) patients with classic acute GVHD and 43 of 193 (43.0%) patients with late acute GVHD (supplemental Table 4). After 1 week of treatment, significantly more patients with late acute GVHD had high MAP scores ($\geq$0.29) than patients with classic acute GVHD (33.7% vs 16.0%; $P < .001$). Patients with high MAP scores after 1 week of treatment had significantly greater risk for NRM than patients with low MAP scores in both classic (at 6 months: 8.3% [95% CI, 6.2-10.7] vs 40.4% [95% CI, 31.1-49.5]; $P < .001$) and late acute GVHD (at 6 months: 5.7% [95% CI, 1.5-14.4] vs 55.5% [95% CI, 33.6-72.8]; $P < .001$; supplemental Figure 2).

Risk factors for late acute GVHD

For patients without a prior history of classic acute GVHD who were alive and without disease relapse on day 100, multivariate analyses showed that advanced recipient age, female donor–to–male recipient sex mismatch, and the use of RIC was significantly associated with an increased risk of late acute GVHD, whereas the use of PTCy-based GVHD prophylaxis significantly reduced the risk of late acute GVHD (Table 5). These risk factors appeared to be associated with shifts in the timing of acute GVHD rather than affecting the overall cumulative incidence of all acute GVHD (supplemental Figure 3). PTCy-based GVHD prophylaxis was

Table 4. Risk factors for NRM 6 months from GVHD treatment

	Without biomarker severity		With biomarker severity	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Time of GVHD onset				
Classic	1	Ref	1	Ref
Late	0.7(2 0.49-1.07)	.100	0.87 (0.53-1.42)	.580
Clinical GVHD severity				
Grades 1/2	1	Ref	1	Ref
Grades 3/4	3.23 (2.44-4.29)	<.001	2.24 (1.59-3.16)	<.001
Biomarker severity				
AA 1			1	Ref
AA 2/3			3.52 (2.48-4.99)	< .001
Recipient age (y), category				
<55	1	Ref	1	Ref
≥55	2.47 (1.64-3.72)	< .001	2.22 (1.42-3.48)	< .001
Sex mismatch				
Other	1	Ref	1	Ref
Female-to-male	1.16 (0.82-1.63)	.400	1.15 (0.78-1.70)	.490
Primary disease				
Acute leukemia	1	Ref	1	Ref
MDS/MPN	1.05 (0.78-1.35)	.870	1.01 (0.70-1.47)	.950
Malignant lymphoma	0.78 (0.46-1.32)	.350	1.06 (0.59-1.88)	.850
Other	1.43 (0.99-2.10)	.060	1.47 (0.92-2.34)	.110
Disease risk				
Standard	1	Ref	1	Ref
High	1.23 (0.88-1.72)	.230	1.03 (0.69-1.52)	.890
Donor type				
HLA-MRD	1	Ref	1	Ref
HLA-MUD	1.20 (0.84-1.70)	.320	1.20 (0.79-1.82)	.400
HLA-MMUD	1.61 (0.92-2.82)	.094	1.87 (0.99-3.55)	.054
Haploidenticaldonor	1.06 (0.48-2.31)	.890	0.86 (0.35-2.08)	.740
GVHD prophylaxis				
CNI and MTX based	1	Ref	1	Ref
CNI and MMF based	0.83 (0.57-1.20)	.310	0.74 (0.49-1.12)	.160
PTCy	0.67 (0.37-1.23)	.200	0.69 (0.37-1.32)	.260
Other	0.83 (0.42-1.65)	.600	0.89 (0.43-1.86)	.760
HCT-CI				
0-2	1	Ref	1	Ref
≥3	1.26 (0.95-1.66)	.110	1.23 (0.89-1.69)	.210
In vivo T-cell depletion				
No	1	Ref	1	Ref
Yes	0.80 (0.58-1.11)	.180	0.75 (0.52-1.07)	.110
Donor source				
Bone marrow	1	Ref	1	Ref
Peripheral blood	0.77 (0.53-1.14)	.190	0.83 (0.55-1.27)	.390
Conditioning				
MAC (TBI < 8 Gy)	1	Ref	1	Ref
MAC (TBI ≥ 8 Gy)	0.89 (0.49-1.62)	.710	0.90 (0.47-1.70)	.730
RIC	1.42 (1.05-1.93)	.024	1.52 (1.06-2.16)	.021
Year of HCT	0.99 (0.92-1.06)	.730	0.97 (0.90-1.06)	.520

Clinical and biomarker severity were evaluated at the time of GVHD treatment.
The number of events in this model was 217.
HCT-CI, HCT-specific comorbidity index; MDS/MPN, myelodysplastic syndromes/myeloproliferative neoplasms; MMF, mycophenolate mofetil; MTX, methotrexate; TBI, total body irradiation.

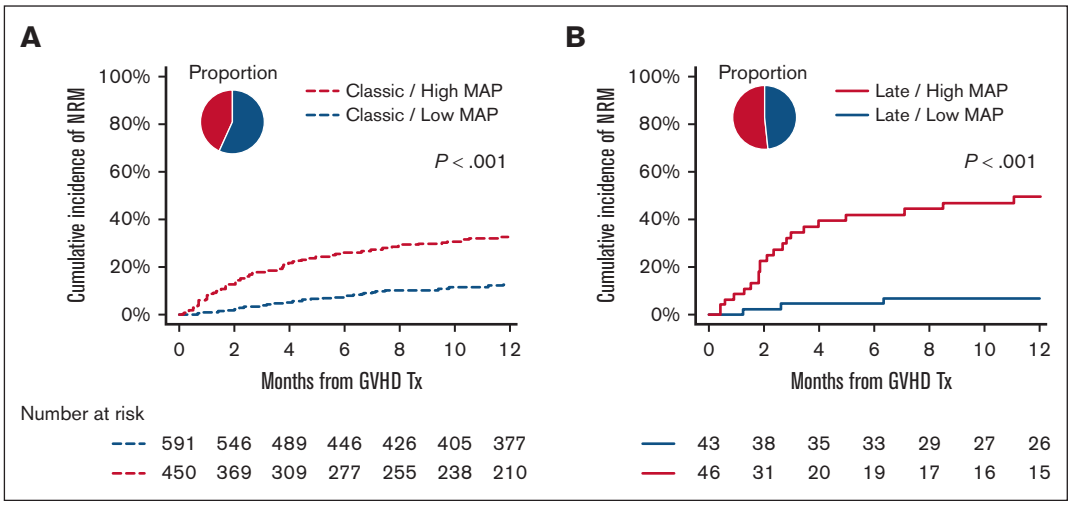

Figure 3. Association of biomarkers with NRM at treatment. (A) The proportion of patients in each risk group in classic acute GVHD at onset of treatment were AA1, 56.8% (591 of 1041) and AA2/3, 43.2% (450 of 1041). The cumulative incidences of NRM at 6 months were 7.4% (95% CI, 5.5-9.8) and 25.9% (95% CI, 22.0-30.1) in AA1 and AA2/3 groups ($P < .001$). (B) The proportion of patients in each risk group in late acute GVHD were 48.3% (43/89) and 51.7% (46/89) for AA1 and AA2/3 groups. The cumulative incidences of NRM at 6 months were 4.9% (95% CI, 0.9-14.7) and 42.2% (95% CI, 27.1-56.6), respectively ($P < .001$).

associated with a significantly earlier onset of acute GVHD, whereas risk factors for late acute GVHD were associated with significantly later onset of GVHD. Because recipient age and conditioning intensity are not independent variables, we performed a multivariate analysis limited to patients treated with a MAC regimen, and advanced recipient age remained an independent risk factor for late acute GVHD (HR, 1.94; 95% CI, 1.08-3.48; $P = .027$).

Discussion

To the best of our knowledge, this is the largest and most comprehensive multicenter study describing the incidence, clinical presentation, risk factors, prognostic value of biomarkers, and outcomes in patients who developed late acute GVHD after HCT. The overall incidence of late acute GVHD that required systemic treatment in our cohort was 5.7%. Although late acute GVHD was more severe at presentation based on both clinical grading and MAP biomarkers when compared with classic acute GVHD, long-term outcomes including NRM, relapse, and OS were not significantly different. Similar to classic acute GVHD, clinical severity and biomarker measurements at presentation were able to accurately risk-stratify patients with late acute GVHD in terms of long-term outcomes.

The Seattle group previously investigated the association of the time of initiation of GVHD treatment with long-term outcomes in patients who received a nonmyeloablative conditioning regimen.[13] They reported that patients who received GVHD treatment (on day 50 or after) had better outcomes than those receiving early GVHD treatment (before day 50) in HLA-matched related HCT, but this was not observed after HLA-matched unrelated HCT. Omer et al and Lee et al also showed that patients with late acute GVHD (n = 7 and n = 26, respectively, in each series) had better survival outcomes than patients with classic acute GVHD, yet definitive conclusions could not be drawn from these findings because of the small sample sizes.[8,14] In this study, our results showed that late acute GVHD was more severe than classic acute GVHD at onset based on both clinical and biomarker parameters and also had a lower overall response rate to initial therapy on day 28. A possible explanation for this difference is that classic acute GVHD onset often occurs during therapeutic levels of immunosuppression, whereas late acute GVHD more often occurs during tapering of immunosuppressive agents or after its discontinuation. Furthermore, patients are monitored less frequently long term after HCT, and a delay in presentation or diagnosis may lead to more severe GVHD upon presentation. Unfortunately, the duration between symptom onset and GVHD diagnosis was not available in our database. Importantly, despite more severe presentation and lower treatment response in late acute GVHD, comparable long-term outcomes in terms of NRM were observed regardless of timing of presentation. Possible explanations for this observation include that concurrent peri-HCT–associated toxicities and immature immune reconstitution may render patients with classic acute GVHD more susceptible to NRM of any cause. Furthermore, patients with late acute GVHD likely represent a healthier population, given that they have survived long enough after HCT to present with late acute GVHD. These explanations are supported by the observation that the HR of NRM in classic acute GVHD was much lower than that in late acute GVHD (2.53 vs 4.37), likely reflecting the declining risk of other causes of NRM, because patients survive longer after HCT.

Risk stratification at the time of acute GVHD onset can potentially guide intensity of immunosuppressive treatment.[25,27,32] We recently reported that treatment with the selective JAK1 inhibitor itacitinib in a low-risk acute GVHD population identified using clinical and biomarker parameters was highly efficacious and caused fewer serious infections than systemic steroids in a case-controlled comparison.[33] This current analysis showed that severity based on clinical or MAP biomarker parameters can accurately risk-stratify patients with late acute GVHD, supporting

Table 5. Risk factors for classic and de novo late acute GVHD

	Classic		Late	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Recipient age (y), category				
<55	1	Ref	1	Ref
≥55	1.00 (0.87-1.16)	.960	1.84 (1.19-2.88)	.007
Sex mismatch				
Other	1	Ref	1	Ref
Female-to-male	0.98 (0.83-1.15)	.780	1.53 (1.09-2.14)	.014
Primary disease				
Acute leukemia	1	Ref	1	Ref
MDS/MPN	1.12 (0.97-1.28)	.120	1.02 (0.73-1.41)	.920
Malignant lymphoma	1.08 (0.87-1.34)	.480	1.00 (0.54-1.84)	.990
Other	1.00 (0.82-1.22)	.530	1.02 (0.61-1.70)	.930
Disease risk				
Standard	1	Ref	1	Ref
High	1.05 (0.91-1.21)	.530	0.89 (0.58-1.35)	.570
Donor type				
HLA-MRD	1	Ref	1	Ref
HLA-MUD	1.56 (1.34-1.81)	< .001	0.77 (0.55-1.07)	.120
HLA-MMUD	1.97 (1.55-2.50)	< .001	0.95 (0.43-2.11)	.910
Haploidentical donor	1.50 (1.14-1.98)	.004	1.27 (0.58-2.81)	.550
GVHD prophylaxis				
CNI and MTX based	1	Ref	1	Ref
CNI and MMF based	1.04 (0.88-1.23)	.650	1.10 (0.66-1.82)	.730
PTCy	1.23 (0.98-1.54)	.066	0.28 (0.13-0.57)	< .001
Other	1.22 (0.92-1.62)	.160	0.62 (0.30-1.32)	.220
HCT-CI				
0-2	1	Ref	1	Ref
≥3	1.01 (0.90-1.14)	.890	1.14 (0.84-1.54)	.400
In vivo T-cell depletion				
No	1	Ref	1	Ref
Yes	0.75 (0.65-0.87)	<.001	0.75 (0.47-1.21)	.240
Donor source				
Bone marrow	1	Ref	1	Ref
Peripheral blood	1.06 (0.90-1.24)	.500	1.26 (0.77-2.08)	.360
Conditioning				
MAC (TBI < 8 Gy)	1	Ref	1	Ref
MAC (TBI ≥ 8 Gy)	1.09 (0.90-1.31)	.370	1.21 (0.65-2.26)	.550
RIC	0.74 (0.65-0.84)	< .001	1.61 (1.13-2.31)	.008
CNI discontinuation by day 100				
Discontinued			1	Ref
Continued			1.00 (0.55-1.84)	.990

The number of events for classic and late acute GVHD was 1245 and 193, respectively.
HCT-CI, HCT-specific comorbidity index; MDS/MPN, myelodysplastic syndromes/myeloproliferative neoplasms; MMF, mycophenolate mofetil; MTX, methotrexate; TBI, total body irradiation.

the inclusion of patients with late acute GVHD in modern risk-stratified trials.[34,35]

To the best of our knowledge, this is the first study large enough to attempt to identify specific risk factors for the development of late acute GVHD. We found that advanced recipient age, female donor–to–male recipient sex mismatch, and the use of RIC were independent risk factors associated with late acute GVHD. Advanced recipient age was associated with late acute GVHD, independent of the conditioning regimen intensity, which might partially explain the relatively higher incidence of late acute GVHD in our study compared with that in the previous studies,[4-6,14] given the large proportion of older patients in our cohort. The recognition of these shifts in the timing of onset of acute GVHD is important because of the rapid increase in use of HCT in older patients worldwide, especially when patients are examined less frequently long term after HCT.[36] In addition, we found that the use of PTCy as GVHD prophylaxis significantly reduced the risk of late acute GVHD, mostly by shifting the timing of acute GVHD to an earlier onset. In contrast, our study indicated that the use of RIC regimens delays the onset of acute GVHD consistent with other reports.[37] It should also be noted that the incidence of late acute GVHD is low, and thus small differences in its incidence translate into large relative effects that have limited clinical significance.

Many studies have reported an association between GVHD and augmented graft-versus-leukemia effects, as evidenced by lower rates of disease relapse in those who develop GVHD.[38-46] We found that classic acute GVHD is indeed associated with lower rates of relapse, but, interestingly, late acute GVHD does not provide such protection in our analysis. Further studies that evaluate relapse risk of individual malignancies are needed to better evaluate the association of late acute GVHD with disease relapse.

Our study has several limitations. Firstly, this analysis included <200 patients with late acute GVHD, so analyses should be interpreted with appropriate caution. For example, the lack of statistical significance for some findings, such as a lack of protective effect from in vivo T-cell depletion for late acute GVHD despite a low HR was the result of underpowered analyses. Secondly, because of less frequent follow-up later after HCT, some mild cases of late acute GVHD that did not require systemic treatment might have been omitted. Thirdly, treatment decisions outside of clinical trials vary because of investigator and center practices. For example, in our cohort, treatment for grade 1 acute GVHD was common, as has been reported in other studies.[47] There was also wide variation in initial steroid dose, as has been observed elsewhere.[48,49] Heterogeneous management of GVHD likely influences outcomes; however, our findings reflect actual practice and, thus, are relevant to the real-word setting. Fourthly, we excluded several subgroups, such as pediatric patients, recipients of umbilical cord blood grafts, and recipients of grafts modified via ex vivo T depletion because of small numbers of patients.

In conclusion, this multicenter, retrospective analysis provides important insights regarding the characteristics and natural history of late acute GVHD. Late acute GVHD is not less severe than classic acute GVHD; in fact its severity at onset appears slightly greater than classic acute GVHD by both clinical and biomarker criteria, although overall outcomes are similar. Late acute GVHD, as defined by traditional day-100 criteria is likely not a distinct entity, and our study provides the rationale for similar treatment intensity as well as the inclusion of late acute GVHD when testing novel treatments.

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Authorship

Contribution: Y.A. designed the study, performed the laboratory analysis, conducted the statistical analysis, and wrote the manuscript; N.S. collected the clinical data, advised statistical methods, and reviewed and revised the manuscript; W.J.H., F.A., Z.D., D.W., H.K.C., E.O.H., W.R., A.M.E., K.S., G.A.Y., C.C., C.L.K., R.R., S.K., M.W., M.E., H.B., M.Q., P.M., S.A.G., P.A.-H., T.S., and E.U. collected the clinical data, and reviewed and revised the manuscript; J.B., S.G., and R.Y. collected and reviewed the clinical data; R.B., S.K., and G.M. performed the laboratory analysis; D.K. advised the statistical analysis; J.E.L., J.L.M.F., and Y.-B.C. 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; N.S., 0000-0001-5274-1749; W.J.H., 0000-0002-5841-4105; Z.D., 0000-0002-7994-8974; E.O.H., 0000-0002-1125-4060; R.R., 0000-0003-2185-9546; H.B., 0000-0002-5190-0899; M.Q., 0000-0001-7689-343X; P.M., 0000-0001-6426-4046; P.A.-H., 0000-0002-0196-806X; E.U., 0000-0001-8530-1192; R.N., 0000-0002-9082-0680; J.E.L., 0000-0002-5611-7828; Y.-B.C., 0000-0002-9554-1058.

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; James L. M. Ferrara, The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Pl, Box 1128, New York, NY 10029; email: james.ferrara@mssm.edu; and Yi-Bin Chen, Hematopoietic Cell Transplant and Cellular Therapy Program, Massachusetts General Hospital, Boston, MA 02114; email: ychen6@partners.org.

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