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Prognostic Biomarkers for Acute Graft-versus-Host Disease Risk after Cyclophosphamide–Fludarabine Nonmyeloablative Allotransplantation

Robert P. Nelson Jr.[1,2], Muhammad Rizwan Khawaja[1], Susan M. Perkins[3], Lindsey Elmore[2], Christen L. Mumaw[2], Christie Orschell[1], Sophie Paczesny[1,2,*]

[1] *Division of Hematology/Oncology, Indiana University School of Medicine and the Melvin and Bren Simon Cancer Center, Indianapolis, Indiana*
[2] *Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana*
[3] *Department of Biostatistics, Indiana University School of Medicine, Indianapolis, Indiana*

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Five candidate plasma biomarkers (suppression of tumorogenesis 2 [ST2], regenerating islet-derived-3α [REG3α], elafin, tumor necrosis factor receptor 1 [TNFR1], and soluble IL-2 receptor-alpha [sIL2Rα]) were measured at specific time points after cyclophosphamide/fludarabine-based nonmyeloablative allotransplantation (NMAT) in patients who did or did not develop acute graft-versus-host disease (aGVHD). Plasma samples from 34 patients were analyzed at days +7, +14, +21, and +30. At a median follow-up of 358 days, 17 patients had experienced aGVHD with a median time to onset at day +36. Risk of aGVHD was associated with elevated plasma ST2 concentrations at day +7 (c-statistic = .72, $P = .03$), day +14 (c-statistic = .74, $P = .02$), and day +21 (c-statistic = .75, $P = .02$); elevated plasma REG3α concentrations at day +14 (c-statistic = .73, $P = .03$), day +21 (c-statistic = .76, $P = .01$), and day +30 (c-statistic = .73, $P = .03$); and elevated elafin at day +14 (c-statistic = .71, $P = .04$). Plasma concentrations of TNFR1 and sIL2Rα were not associated with aGVHD risk at any of the time points studied. This study identified ST2, REG3α, and elafin as prognostic biomarkers to evaluate risk of aGVHD after cyclophosphamide/fludarabine-based NMAT. These results need to be confirmed in an independent validation cohort.

INTRODUCTION

Acute graft-versus-host disease (aGVHD) continues to be a major contributor to early transplant-related mortality after allogeneic hematopoietic cell transplantation. There is no reliable way to determine before the onset of symptoms who will suffer complications. To date, the choice of candidate biomarkers for aGVHD has been guided by studies performed in groups of patients who received myeloablative full or reduced-intensity conditioning. We previously demonstrated that a biomarker panel consisting of IL-2 receptor-alpha (IL2Rα), tumor necrosis factor receptor-1 (TNFR1), IL-8, and hepatocyte growth factor correlated with clinical diagnosis of aGVHD as well as survival, independent of clinical grade severity. A panel of 6 biomarkers predicted treatment response and survival after aGVHD [1,2]. Recently, the suppression of tumorogenesis 2 (ST2) was identified as a novel marker useful in predicting glucocorticoid-resistant aGVHD and nonrelapse mortality (NRM) [3].

Nonmyeloablative allotransplantation (NMAT) conditioning extends allotransplant options to older individuals who may be at higher risk for aGVHD on the basis of age; NMAT, a minimally intense RIC is associated with low incidences of early transplant-related complications and mortality. Cyclophosphamide (Cy) and fludarabine (Flu) based NMAT enables engraftment in recipients of related and unrelated HLA-matched grafts without mucositis and/or sinusoidal obstructive syndrome [4,5]. The validation of biomarkers across a variety of settings is critical before attempting to integrate their use in clinical practice. We conducted a study to test the ability of plasma levels of 5 individual biomarkers at specific time points to serve as prognostic markers for aGVHD among patients undergoing Cy/Flu-based NMAT.

METHODS

Patient Population

Thirty-four patients with hematological malignancies who underwent Cy/Flu-based NMAT at Indiana University between 2008 and 2012 were included in the study, which was approved by the Indiana University institutional review board. Disease status at transplant was categorized according to the American Society of Blood and Marrow Transplantation criteria [6].

Patients received mobilized peripheral blood hematopoietic cells from matched related or matched unrelated donors. GVHD prophylaxis for matched unrelated donor recipients consisted of cyclosporine A ± mycophenolate mofetil or basiliximab (NCT00975975) or a combination of tacrolimus and sirolimus. Matched related recipients received a combination of cyclosporine A ± mycophenolate mofetil or basiliximab. Patients were followed prospectively until death or for a median of 358 days (range, 182 to 1381 days) and

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* Correspondence and reprint requests: Sophie Paczesny, MD, PhD, Department of Pediatrics, Bone Marrow and Stem Cell Transplantation Program, Indiana University Melvin and Bren Simon Cancer Center and Wells Center for Pediatric Research, 1044 W Walnut Street, Rm 425, Indianapolis, IN 46202.
E-mail address: sophpacz@iu.edu (S. Paczesny).

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Table 1
Patient Characteristics

	Total (N = 34)	aGVHD+ (n = 17)	No aGVHD (n = 17)
Median age, yr (range)	60 (29-72)	61 (29-72)	59 (33-66)
Diagnosis			
Acute leukemia	14	8	6
Chronic leukemia	5	4	1
MDS/MF	9	3	6
NHL	4	1	3
HD	2	1	1
Donor			
Related	16	4	12
Unrelated	18	13	5
Match			
Fully matched	32	15	17
Mismatched	2	2	0
GVHD prophylaxis			
Cyclosporine A/basiliximab	16	10	6
Tacrolimus/sirolimus	15	6	9
Cyclosporine A/mycophenolate mofetil	3	1	2
ASBMT status*			
Low risk	11	5	6
Intermediate risk	14	8	6
High risk	8	3	5

MDS indicates myelodysplastic syndrome; MF, myelofibrosis; NHK, non-Hodgkin lymphoma; HD, Hodgkin disease; ASBMT, American Society of Blood and Marrow Transplantation.

* Not applicable for one subject with myelofibrosis.

divided into aGVHD+ and no aGVHD groups. Modified Glucksberg criteria were used to diagnose and grade aGVHD at onset and at maximum severity [7]. Histopathological confirmation of aGVHD was obtained whenever clinically feasible.

Sample Preparation and Processing

Ten to 20 mL of whole blood was obtained from patients on days +7, +14, +21, and +30 in heparin-containing tubes to prevent clotting. Plasma was obtained from blood samples by centrifugation. Samples were aliquoted without additives into cryovials and stored at −80°C.

Five plasma biomarkers were studied: ST2, regenerating islet-derived-3α (REG3α), elafin, TNFR1, and soluble IL2Rα (sIL2Rα). Plasma ST2, elafin, and TNFR1 levels were measured by ELISA using commercially available kits (DST200, DY1747, and DY225, respectively; R&D Quantikine, Minneapolis, MN). Plasma REG3α was measured using ELISA kit 5323 (MBL International Corp., Woburn, MA), and sIL2Rα was measured using a commercially available multiplex platform (MPXHCYTO-60K; Millipore Corp., Billerica, MA). All assays were performed in compliance with protocols provided by kit manufacturers.

Statistical Analysis

Differences in patient characteristics between aGVHD+ and no aGVHD groups were determined using Wilcoxon rank sum test for age at transplant and Fisher's exact test for all categorical variables. Medians and 25th and 75th percentiles of individual biomarker levels in aGVHD+ and no aGVHD groups were calculated and distributions compared at each time point using exact Wilcoxon rank sum tests (because of non-normality of biomarkers), and corresponding c-statistics, which represent the area under the receiver operating characteristic curves, were calculated by fitting logistic regression models. Biomarkers with statistically significant prognostic value were further analyzed to determine their ability to predict grades III to IV or gastrointestinal (GI)-specific aGVHD (versus no aGVHD using exact Wilcoxon test). Association of elevated (median or higher) biomarker levels with overall survival and NRM was determined using log-rank test and Gray's test. $P \leq .05$ was considered to be the criteria of statistical significance. All statistical analyses were performed in SAS version 9.3 (SAS Institute, Cary, NC).

Table 2
aGVHD Grade at Onset and at Maximum

	aGVHD Grade			
	I	II	III	IV
At onset				
Overall	3	9	5	0
Skin	1	4	3	0
GI	6	1	4	0
Liver	2	2	1	0
At maximum				
Overall	2	5	8	2
Skin	0	3	6	0
GI	3	1	6	2
Liver	2	3	1	0

RESULTS

Of 34 patients included in the study, 17 experienced aGVHD and 17 did not. Table 1 describes patient characteristics. Age, diagnosis, match, GVHD prophylaxis, and American Society of Blood and Marrow Transplantation risk did not differ between aGVHD+ and no aGVHD groups ($P > .16$ for each). Patients who received grafts from an unrelated donor developed aGVHD more frequently (76% versus 24%; $P = .015$). Median onset of aGVHD was day +36 (range, +17 to 151). Table 2 describes the overall and site-specific severity of aGVHD at onset and at maximum clinical grade according to modified Glucksburg criteria. Of 17 patients who experienced aGVHD, 9 had skin, 12 had GI, and 6 had liver involvement.

Table 3 shows the median and 25th and 75th percentiles of plasma biomarker concentrations in aGVHD+ and no aGVHD groups at specific time points after hematopoietic cell transplantation and corresponding c-statistics and *P* values. Elevated plasma ST2 levels at days +7, +14, and +21 were significant risk factors for aGVHD occurrence (Figure 1A). Similarly, plasma REG3α levels at days +14, +21, and +30 were also significantly elevated among aGVHD+ patients (Figure 1B). Elevated plasma elafin levels at day +14 were also associated with aGVHD. When ST2 and REG3α were considered together in a logistic regression model, the corresponding c-statistics were .68 at day +7, .77 at day +14, .75 at day +21, and .74 at day +30.

We compared sIL2Rα levels using exact Wilcoxon tests in patients who received or did not receive basiliximab. Those

Table 3
Biomarker Medians (25th and 75th percentile in parentheses) by Group Over Time*

	Day +7				Day +14	
	aGVHD+	No aGVHD	c-statistic	*P*	aGVHD+	No aGVHD
ST2, ng/mL	73.2 (25.9, 149.2)	25.7 (12.9, 42.7)	.72	.03	53.8 (26.8, 95.9)	19.8 (14.4, 42.3)
Reg3α, pg/mL	51.4 (24.2, 93.5)	40.5 (20.0, 53.2)	.63	.20	194.8 (46.7, 825.0)	50.3 (23.6, 72.9)
Elafin, pg/mL	7628.3 (4303.0, 18557.2)	5088.0 (3530.3, 8057.8)	.62	.26	10156.8 (5531.0, 14428.2)	4610.0 (3416.2, 8140.6)
TNFR1, pg/mL	4348.9 (2408.7, 6562.1)	3747.1 (2025.1, 4341.2)	.62	.23	5695.1 (2946.4, 9701.1)	3809.8 (2391.9, 4935.8)
sIL2Rα, pg/mL	883.2 (254.6, 1673.0)	137.5 (50.1, 481.0)	.70	.14	269.1 (26.1, 3051.3)	95.7 (83.8, 265.3)

* For ST2, Reg3α, elafin, and TNFR1, sample sizes were 17, 16, 17, and 16 for aGVHD+ and 17, 15, 15, and 16 for no aGVHD for days +7, +14, +21, and +30, respectively. For sIL2Rα, sample sizes were 12, 10, and 10 for aGVHD+ and 10, 13, and 14 for no aGVHD for days +7, +14, and +21 respectively.

who received basiliximab had higher values of sIL2Rα on day +7 ($P = .003$), lower values on day +14 ($P = .02$), and no difference in values on day +21 ($P = .27$).

The difference of day +14 biomarker levels between grades III to IV aGVHD$^+$ and no aGVHD was not statistically significant: median ST2 34.0 and 19.8 ng/mL ($P = .10$), median REG3α 180.9 and 50.3 pg/mL ($P = .07$), and median elafin 7818.1 and 4610.0 pg/mL ($P = .07$), respectively. The difference of day +14 REG3α levels between GI aGVHD$^+$ and no aGVHD also did not reach statistical significance: median 208.7 versus 50.3 pg/mL ($P = .08$).

Elevated (≥50th percentile) biomarker levels at day +14 were not associated with overall survival: ST2 median 792 versus 442 days (log-rank $P = .824$), REG3α 792 versus 442 days ($P = .558$), and elafin 1081 versus 545 days ($P = .582$). Similarly, elevated biomarker levels at day +14 were also not associated with NRM.

DISCUSSION

This study was conducted to determine prognostic plasma biomarkers for aGVHD after Cy/Flu-based NMAT. ST2, REG3α, and elafin levels were elevated at certain time points in patients who developed aGVHD versus those who did not. These were, however, not significant prognostic biomarkers for other endpoints, including grades III to IV aGVHD, overall survival and NRM, possibly because sample size of the study was not powered for these endpoints.

The choice of biomarkers to consider was based on previous findings in cohorts undergoing myeloablative allotransplantation [2,3,8,9]. REG3α is secreted by Paneth cells into intestinal crypts and reduces inflammation, protects intestinal stem cells, and prevents GI epithelial damage [10]. REG3α does not mediate aGVHD but appears to protect damaged epithelium. It is a biomarker for GVHD of the GI tract and a predictor of NRM [11]. Day +14 REG3α levels were not statistically higher in patients with GI aGVHD compared with no aGVHD, which we believe is attributable to the relatively small number of patients in this study. Elafin is associated with severity and mortality from aGVHD of the skin [8]. As part of a panel consisting of 6 biomarkers, REG3α and elafin predicted treatment response and survival from aGVHD [2]. ST2 in its soluble form acts as a negative regulator of type 2 helper T cells [12]. ST2 was recently identified as a marker that predicted glucocorticoid-resistant aGVHD and NRM [3].

In the present study, it is noteworthy that we did not find TNFR1 or sIL2Rα elevated in those who developed aGVHD and elafin was significantly elevated at only day +14. The relatively small sample size might not yield sufficient power to detect the prognostic value of all relevant biomarkers. Also, the use of prognostic markers identified in the setting of higher intensity conditioning might not be applicable

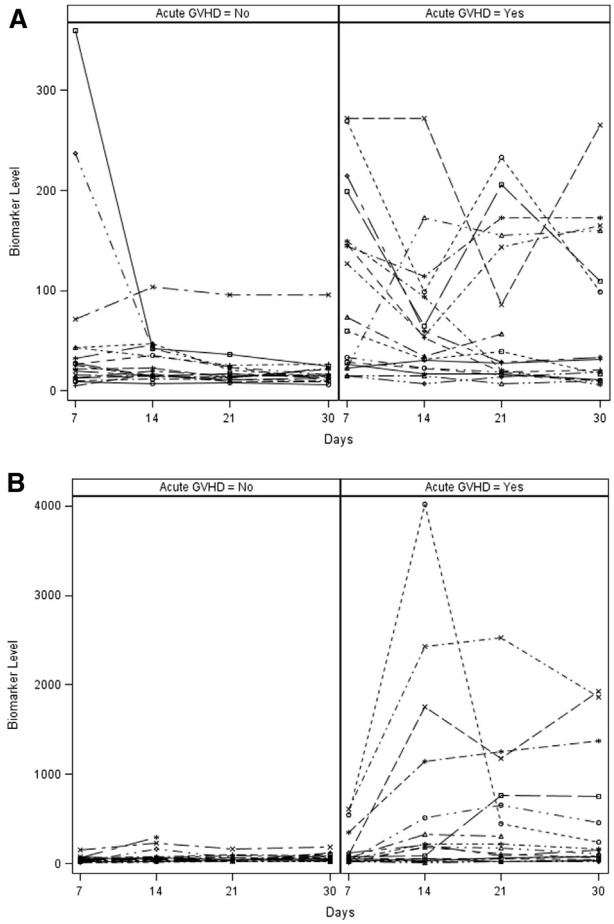

Figure 1. (A) ST2 (ng/mL) levels over time for individual patients in aGVHD$^+$ and no aGVHD groups. (B) REG3α (pg/mL) levels over time for individual patients in aGVHD$^+$ and no aGVHD groups.

during Cy/Flu-based NMAT. Finally, it is plausible that using basiliximab (given at day +7, +8, or +9) as part of GVHD prophylaxis for some patients led to lower levels of day +14 sIL2Rα and confounded results; subset analysis among patients not receiving basiliximab was not done because of the relatively small number of patients. The higher level of sIL2Rα at day +7 was obtained before basiliximab administration, when cyclosporine was the only immunosuppressive present in the circulation.

Biomarkers are most valuable if they identify patients at high risk of an adverse outcome before the clinical signs are apparent. This could potentially provide clinicians with sufficient time to institute appropriate interventions before significant tissue damage has occurred and hopefully avert the adverse outcome. For instance, patients at high risk of

Table 3
(*continued*)

Day +14		Day +21			Day +30			
c-statistic	*P*	aGVHD$^+$	No aGVHD	c-statistic *P*	aGVHD$^+$	No aGVHD	c-statistic	*P*
.74	.02	28.3 (18.8, 143.9)	16.2 (12.5, 23.7)	.75 .02	26.3 (10.3, 134.4)	15.0 (11.2, 22.0)	.68	.09
.73	.03	178.1 (54.0, 649.5)	41.7 (29.6, 64.6)	.76 .01	130.6 (52.5, 605.2)	51.4 (30.1, 87.8)	.73	.03
.71	.04	7793.0 (4610.0, 10586.5)	5931.4 (3916.6, 10941.0)	.54 .74	6987.6 (5355.0, 12224.5)	8794.1 (5786.9, 15783.8)	.57	.45
.64	.20	5443.2 (3974.0, 6784.1)	4528.0 (2692.6, 5492.5)	.67 .11	4421.9 (3261.4, 7989.6)	4094.6 (3150.5, 5572.2)	.49	.33
.61	.41	930.5 (194.3, 2098.1)	216.6 (167.4, 398.1)	.68 .13				

developing aGVHD may be started on treatment preemptively. Among biomarkers identified in current study, only ST2 was prognostic of aGVHD risk before day +14 after hematopoietic cell transplantation; this is not surprising given the relatively late (median, 36 days) onset of aGVHD after NMAT. Clinically relevant prognostic tools proposed in prior studies consisted of a panel rather than a single biomarker; therefore, combinations of biomarkers need to be explored further [1,2,9].

In conclusion, the current study identified ST2, REG3α, and elafin as prognostic biomarkers to stratify for risk of developing aGVHD after Cy/Flu-based NMAT. These results need to be confirmed in a large independent validation cohort, ideally among a number of institutions, to establish clinically useful cut-offs for their future use in clinical trials.

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Conflict of interest statement: S.P. holds a patent on "methods of detection of graft-versus-host disease" (US Patent 13/573,766).

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Brentuximab Vedotin Is Associated with Improved Progression-Free Survival after Allogeneic Transplantation for Hodgkin Lymphoma

Robert Chen [1,*], Joycelynne M. Palmer [2], Ni-Chun Tsai [2], Sandra H. Thomas [1], Tanya Siddiqi [1], Leslie Popplewell [1], Len Farol [3], Auayporn Nademanee [1], Stephen J. Forman [1]

[1] *Department of Hematology/Hematopoietic Cell Transplantation, City of Hope, Duarte, California*
[2] *Division of Biostatistics, City of Hope, Duarte, California*
[3] *Bone Marrow Transplantation Program, City of Hope–Kaiser Permanente, Los Angeles, California*

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We previously reported that brentuximab vedotin (BV) enabled successful reduced-intensity allogeneic hematopoietic cell transplantation (RIC-alloHCT) in patients with relapsed Hodgkin lymphoma, after a median follow-up of 14.4 months. We now provide an updated report on 21 patients who were treated from 2009 to 2012 with BV before RIC-alloHCT with a uniform fludarabine/melphalan conditioning regimen and donor source after a median follow-up of 29.9 months. We have also retrospectively compared the patient characteristics and outcomes of these BV-pretreated patients to 23 patients who received fludarabine/melphalan RIC-alloHCT without prior BV, in the time period before the drug was available (2003 to 2009). Patients who were treated with BV before RIC-alloHCT had a lower median hematopoietic cell transplantation–specific comorbidity index and a reduced number of peri-transplantation toxicities. There were also improvements in 2-year progression-free survival (59.3% versus 26.1%) and cumulative incidence of relapse/progression (23.8% versus 56.5%).

INTRODUCTION

Brentuximab vedotin (BV) is an antibody-drug conjugate of anti-CD30 antibody and the microtubule-disrupting agent, monomethyl auristatin E [1]. BV is approved for use in Hodgkin lymphoma (HL) patients who have failed autologous hematopoietic cell transplantation (autoHCT). Phase II studies report

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* Correspondence and reprint requests: Robert Chen, MD, Department of Hematology/HCT, City of Hope, 1500 E. Duarte Road, Duarte, CA 91010.
E-mail address: rchen@coh.org (R. Chen).

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