SUPPLEMENTARY APPENDIX

Plasma-derived proteomic biomarkers in human leukocyte antigen-haploidentical or human leukocyte antigen-matched bone marrow transplantation using post-transplantation cyclophosphamide

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 doi:10.3324/haematol.2016.152322

Received: July 8, 2016.

Accepted: January 20, 2017.

Pre-published: January 25, 2017.

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Supplementary Materials for

Plasma-Derived Proteomic Biomarkers in HLA-Haploidentical or HLA-Matched Bone Marrow Transplantation Using Post-Transplantation Cyclophosphamide

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This PDF file includes:

Supplementary Methods

Proteomic analysis

The antibody pairs included: REG3α (MBL International, Woburn, MA, USA; Ab-Match Assembly Human PAP1 [REG3α] kit and Ab-Match Universal kit) and CXCL9 (RayBiotech, Norcross, GA, USA; RayBio Human MIG ELISA Kit). Duoset kits were used for IL-2Rα, IL-6, TNFR-1, and elafin, and the quantikine kit was used for ST2 (R&D Systems, Minneapolis, MN, USA). All the kits permitted comparable measurements in plasma or serum, and thus the ST2 Duoset kit was not used for this study. Samples were analyzed in duplicate as previously described.[1] Pipetting for the REG3α assay (384-well plate format) was performed using the EpMotion4500 liquid handling system (Eppendorf, Hauppauge, NY, USA) and for other assays (96-well plate format) by multichannel or the Multidrop™ 384 Reagent Dispenser (ThermoFisher Scientific, Waltham, MA, USA). All washes were performed using the Aquamax 2000 plate washer (Molecular Devices, Sunnyvale, CA, USA). Absorbance was measured immediately after termination of the substrate reaction using a SpectraMax Plus plate reader (Molecular Devices), and results were calculated using SoftMax Pro, version 6.2.2 (Molecular Devices). Laboratory investigators were blinded to all clinical information and transplant outcomes.

Definitions and endpoints

Pre-transplant remission status was determined according to standard criteria.[2] Acute and chronic graft-versus-host disease (GVHD) also were diagnosed and graded using standard criteria.[3, 4] Patients who experienced graft failure were excluded from these analyses. Therefore,

non-relapse mortality (NRM), relapse, and donor lymphocyte infusion were the only competing risks for chronic GVHD. NRM, relapse, donor lymphocyte infusion, and chronic GVHD were competing risks for acute GVHD. Relapse was defined as any detectable disease post-transplant, even at a flow cytometric, molecular, or cytogenetic level. Relapse was a competing risk for NRM and vice versa.

The starting time for all patients for NRM and GVHD outcomes was the date of transplant. For acute GVHD, those with onset prior to or on day 30 were excluded (all patients, n=31, 19.6%; HLA-matched cohort, n=26, 26%; HLA-haploidentical cohort, n=5, 8.6%). For chronic GVHD, those with onset prior to or on day 182 were excluded (all patients, n=10, 6.3%; HLA-matched cohort, n=8, 8%; HLA-haploidentical cohort, n=2, 3.5%) as were those with competing risk events (all patients, n=23, 15%; HLA-matched cohort, n=14, 14%; HLA-haploidentical cohort, n=9, 16%).

References

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4. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11(12):945-956.

Table S1. Organ distributions of acute GVHD.

	HLA-Matched Cohort			HLA-Haploidentical Cohort		
	Skin	**GI Tract**	**Liver**	**Skin**	**GI Tract**	**Liver**
Number of Patients (%)	42 (42%)	39 (39%)	13 (13%)	7 (12%)	7 (12%)	0 (0%)

Abbreviations: HLA, human leukocyte antigen; GVHD, graft-versus-host disease; GI, gastrointestinal.

Table S2. Univariable associations of day 30 biomarker levels with acute GVHD and non-relapse mortality adjusted for the age and cytomegalovirus serostatus of the recipient.

	Grade II-IV Acute GVHD		Grade III-IV Acute GVHD		Non-Relapse Mortality	
Biomarker	**SHR (95% CI)**	**p-value**	**SHR (95% CI)**	**p-value**	**SHR (95% CI)**	**p-value**
IL-2Rα (per 500 units)						
HLA-matched	**1.30 (1.07-1.57)**	**0.009**	**1.45 (1.16-1.82)**	**0.001**	**1.52 (1.14-2.03)**	**0.005**
HLA-haploidentical	**1.69 (1.03-2.75)**	**0.037**	**1.60 (1.01-2.53)**	**0.047**	1.65 (0.98-2.80)	0.06
TNFR-1 (per 1000 units)						
HLA-matched	1.04 (0.95-1.13)	0.40	1.08 (0.98-1.19)	0.15	**1.20 (1.07-1.34)**	**0.002**
HLA-haploidentical	1.00 (0.90-1.11)	0.96	1.00 (0.85-1.17)	0.98	**1.40 (1.27-1.54)**	**<0.001**
ST2 (per 20 units)						
HLA-matched	1.05 (0.94-1.16)	0.42	1.05 (0.87-1.27)	0.62	**1.14 (1.04-1.25)**	**0.005**
HLA-haploidentical	1.10 (0.91-1.34)	0.33	1.11 (0.88-1.38)	0.38	**1.43 (1.30-1.58)**	**<0.001**
REG3α (per 20 units)						
HLA-matched	0.99 (0.97-1.02)	0.55	1.01 (0.99-1.03)	0.46	**1.05 (1.03-1.08)**	**<0.001**
HLA-haploidentical	0.99 (0.98-1.00)	0.12	0.99 (0.97-1.01)	0.20	**1.01 (1.01-1.02)**	**<0.001**
Elafin (per 1000 units)						
HLA-matched	1.03 (0.98-1.08)	0.30	1.00 (0.95-1.06)	0.98	1.00 (0.93-1.06)	0.88
HLA-haploidentical	1.06 (0.97-1.15)	0.19	1.01 (0.95-1.08)	0.68	**1.07 (1.01-1.14)**	**0.019**
IL-6 (per 10 units)						
HLA-matched	1.00 (0.99-1.01)	0.95	1.00 (0.99-1.01)	0.90	1.00 (0.98-1.01)	0.47
HLA-haploidentical	0.97 (0.82-1.13)	0.67	**0.10 (0.01-0.71)**	**0.02**	**1.05 (1.03-1.07)**	**<0.001**
CXCL9 (per 5 units)						
HLA-matched	1.03 (0.99-1.07)	0.21	1.03 (0.99-1.07)	0.17	1.03 (0.97-1.09)	0.28
HLA-haploidentical	1.45 (0.89-2.37)	0.13	**2.55 (1.62-4.02)**	**<0.001**	0.93 (0.54-1.60)	0.79

Notes: For grade II-IV acute GVHD, the numbers of events and competing risks were 31 and 23 for the HLA-matched cohort and 6 and 19 for the HLA-haploidentical cohort, respectively. For grade III-IV acute GVHD, the numbers of events and competing risks were 16 and 39 for the HLA-matched cohort and 2 and 23 for the HLA-haploidentical cohort, respectively. For non-relapse mortality, the numbers of events and competing risks were 14 and 37 for the HLA-matched cohort and 10 and 12 for the HLA-haploidentical cohort, respectively. Biomarkers for these analyses were assessed as continuous variables. The SHR listed for each biomarker/outcome is the risk per given number of biomarker units shown. Abbreviations: GVHD, graft-versus-host disease; IL-2Rα, interleukin-2 receptor alpha; TNFR-1, tumor necrosis factor receptor-1; ST2, serum stimulation-2 (IL1RL1 gene product); REG3α, regenerating islet-derived 3-alpha; IL-6, interleukin-6; CXCL9, chemokine [C-X-C motif] ligand 9; HLA, human leukocyte antigen; SHR, subdistribution hazard ratio; CI, confidence interval.

Table S3. Univariable analyses for the HLA-matched cohort using day 30 biomarker levels dichotomized at the median.

	Non-Relapse Mortality			Relapse			Grade II-IV Acute GVHD			Grade III-IV Acute GVHD			Chronic GVHD		
Biomarker	**CIF**	**95% CI**	**p-value**	**CIF**	**95% CI**	**p-value**	**CIF**	**95% CI**	**p-value**	**CIF**	**95% CI**	**p-value**	**CIF**	**95% CI**	**p-value**
IL-2ra			0.017			0.158			0.038			0.006			0.142
< 1835	0	-		0.116	(0.043, 0.231)		0.267	(0.126, 0.430)		0.048	(0.009, 0.142)		0.14	(0.057, 0.259)	
≥ 1835	0.158	(0.078, 0.263)		0.228	(0.130, 0.343)		0.523	(0.367, 0.657)		0.269	(0.158, 0.393)		0.035	(0.007, 0.107)	
TNFR-1			0.009			0.282			0.023			0.008			0.259
< 4433	0.019	(0.002, 0.088)		0.075	(0.024, 0.166)		0.3	(0.168, 0.444)		0.075	(0.024, 0.166)		0.113	(0.046, 0.214)	
≥ 4433	0.17	(0.080, 0.289)		0.298	(0.176, 0.430)		0.559	(0.378, 0.706)		0.293	(0.164, 0.434)		0.043	(0.008, 0.128)	
ST2			0.69			0.211			0.358			0.403			0.959
< 42	0.082	(0.030, 0.167)		0.131	(0.061, 0.228)		0.366	(0.223, 0.510)		0.14	(0.066, 0.243)		0.098	(0.040, 0.188)	
≥ 42	0.103	(0.033, 0.220)		0.256	(0.133, 0.399)		0.485	(0.308, 0.641)		0.216	(0.102, 0.358)		0.051	(0.009, 0.152)	
REG3a			0.011			0.193			0.238			0.637			0.074
< 56	0.022	(0.002, 0.100)		0.152	(0.067, 0.270)		0.357	(0.217, 0.499)		0.152	(0.067, 0.270)		0.13	(0.053, 0.244)	
≥ 56	0.148	(0.069, 0.255)		0.204	(0.109, 0.319)		0.5	(0.319, 0.657)		0.188	(0.093, 0.308)		0.037	(0.007, 0.113)	
Elafin			0.442			0.361			0.219			0.414			0.806
< 9619	0.079	(0.020, 0.191)		0.211	(0.099, 0.350)		0.5	(0.306, 0.666)		0.132	(0.048, 0.258)		0.132	(0.048, 0.258)	
≥ 9619	0.097	(0.039, 0.185)		0.161	(0.083, 0.263)		0.37	(0.234, 0.506)		0.196	(0.105, 0.309)		0.048	(0.013, 0.122)	
IL-6			0.28			0.852			0.026			0.011			0.554
< 32	0.059	(0.011, 0.172)		0.147	(0.054, 0.285)		0.2	(0.062, 0.393)		0.031	(0.002, 0.137)		0.088	(0.023, 0.211)	
≥ 32	0.106	(0.047, 0.194)		0.197	(0.112, 0.300)		0.5	(0.361, 0.624)		0.242	(0.144, 0.353)		0.076	(0.028, 0.155)	
CXCL9			0.016			0.51			0.032			0.029			0.328
< 6	0.058	(0.015, 0.144)		0.135	(0.059, 0.241)		0.278	(0.145, 0.428)		0.083	(0.027, 0.182)		0.077	(0.025, 0.169)	
≥ 6	0.125	(0.051, 0.234)		0.229	(0.123, 0.355)		0.553	(0.383, 0.693)		0.261	(0.145, 0.392)		0.083	(0.027, 0.182)	

Notes: Day 30 post-transplant biomarker values were used for these analyses. The CIFs were assessed at 180 days post-transplant. The p-values are based on the non-parametric Gray test for the comparison of cumulative incidence curves. (It is not a test for comparison at 180 days. Rather, it compares the whole cumulative incidence functions.) CIF = 0 indicates no events within the first 6 months.
Abbreviations: GVHD, graft-versus-host disease; CIF, cumulative incidence function; IL, interleukin; TNFR-1, tumor necrosis factor receptor-1; ST2, serum stimulation-2 (IL1RL1 gene product); REG3α, regenerating islet-derived 3-alpha; CXCL9, chemokine (C-X-C motif) ligand 9.

Table S4. Univariable analyses for the HLA-haploidentical cohort using day 30 biomarker levels dichotomized at the median.

	Non-Relapse Mortality			Relapse			Grade II-IV Acute GVHD			Grade III-IV Acute GVHD			Chronic GVHD		
Biomarker	**CIF**	**95% CI**	**p-value**	**CIF**	**95% CI**	**p-value**	**CIF**	**95% CI**	**p-value**	**CIF**	**95% CI**	**p-value**	**CIF**	**95% CI**	**p-value**
IL-2ra			0.002			0.235			0.086			0.059			0.656
< 1835	0.056	(0.010, 0.163)		0.028	(0.002, 0.124)		0.029	(0.002, 0.130)		0	-		0.028	(0.002, 0.124)	
≥ 1835	0.318	(0.142, 0.511)		0	-		0.211	(0.066, 0.410)		0.1	(0.017, 0.272)		0.045	(0.003, 0.189)	
TNFR-1			0.014			0.599			0.509			0.907			0.961
< 4433	0	-		0.038	(0.003, 0.164)		0.083	(0.014, 0.233)		0.04	(0.003, 0.170)		0.038	(0.003, 0.164)	
≥ 4433	0.281	(0.140, 0.441)		0	-		0.103	(0.026, 0.243)		0.033	(0.002, 0.145)		0.031	(0.002, 0.137)	
ST2			0.021			0.014			0.461			0.34			0.065
< 42	0	-		0.056	(0.004, 0.224)		0.063	(0.004, 0.247)		0	-		0.111	(0.019, 0.298)	
≥ 42	0.225	(0.112, 0.363)		0	-		0.108	(0.034, 0.230)		0.053	(0.010, 0.155)		0	-	
REG3a			0.001			0.57			0.104			0.081			0.558
< 56	0.03	(0.002, 0.134)		0	-		0.03	(0.002, 0.134)		0	-		0.03	(0.002, 0.134)	
≥ 56	0.32	(0.152, 0.502)		0.04	(0.003, 0.170)		0.2	(0.062, 0.393)		0.091	(0.016, 0.251)		0.04	(0.003, 0.170)	
Elafin			0.001			0.066			0.146			0.458			0.879
< 9619	0.049	(0.009, 0.145)		0.024	(0.002, 0.110)		0.051	(0.009, 0.152)		0.025	(0.002, 0.113)		0.024	(0.002, 0.110)	
≥ 9619	0.412	(0.186, 0.626)		0	-		0.214	(0.052, 0.448)		0.067	(0.004, 0.260)		0.059	(0.004, 0.235)	
IL-6			0.16			0.897			0.084			0.007			0.85
< 32	0.133	(0.054, 0.249)		0.022	(0.002, 0.101)		0.049	(0.009, 0.145)		0	-		0.044	(0.008, 0.133)	
≥ 32	0.231	(0.056, 0.475)		0	-		0.25	(0.060, 0.505)		0.167	(0.027, 0.413)		0	-	
CXCL9			0.064			0.419			0.068			0.132			0.561
< 6	0.037	(0.003, 0.159)		0	-		0.16	(0.050, 0.325)		0.077	(0.013, 0.217)		0.037	(0.003, 0.159)	
≥ 6	0.258	(0.122, 0.418)		0.032	(0.002, 0.141)		0.036	(0.003, 0.154)		0	-		0.032	(0.002, 0.141)	

Notes: Day 30 post-transplant biomarker values were used for these analyses. The CIFs were assessed at 180 days post-transplant. The p-values are based on the non-parametric Gray test for the comparison of cumulative incidence curves. (It is not a test for comparison at 180 days. Rather, it compares the whole cumulative incidence functions.) CIF = 0 indicates no events within the first 6 months.
Abbreviations: GVHD, graft-versus-host disease; CIF, cumulative incidence function; IL, interleukin; TNFR-1, tumor necrosis factor receptor-1; ST2, serum stimulation-2 (IL1RL1 gene product); REG3α, regenerating islet-derived 3-alpha; CXCL9, chemokine (C-X-C motif) ligand 9.

Table S5. Causes of death.

HLA-MATCHED COHORT (n=14 NRM EVENTS)	
PRIMARY CAUSE	**Number (% of NRM events in cohort)**
Acute GVHD	6 (43%)
Chronic GVHD	2 (14%)
Infection	2 (14%)
Diffuse alveolar hemorrhage	1 (7%)
Adult respiratory distress syndrome	1 (7%)
Multi-organ system failure	1 (7%)
Veno-occlusive disease	1 (7%)
HLA-HAPLOIDENTICAL COHORT (n=10 NRM EVENTS)	
PRIMARY CAUSE	**Number (% of NRM events in cohort)**
Multi-organ system failure	3 (30%)
Infection	2 (20%)
Diffuse alveolar hemorrhage	1 (10%)
Veno-occlusive disease	1 (10%)
Calcineurin-inhibitor-related microangiopathy	1 (10%)
Unknown	2 (20%)

Abbreviations: HLA, human leukocyte antigen; NRM, non-relapse morality; GVHD, graft-versus-host disease.

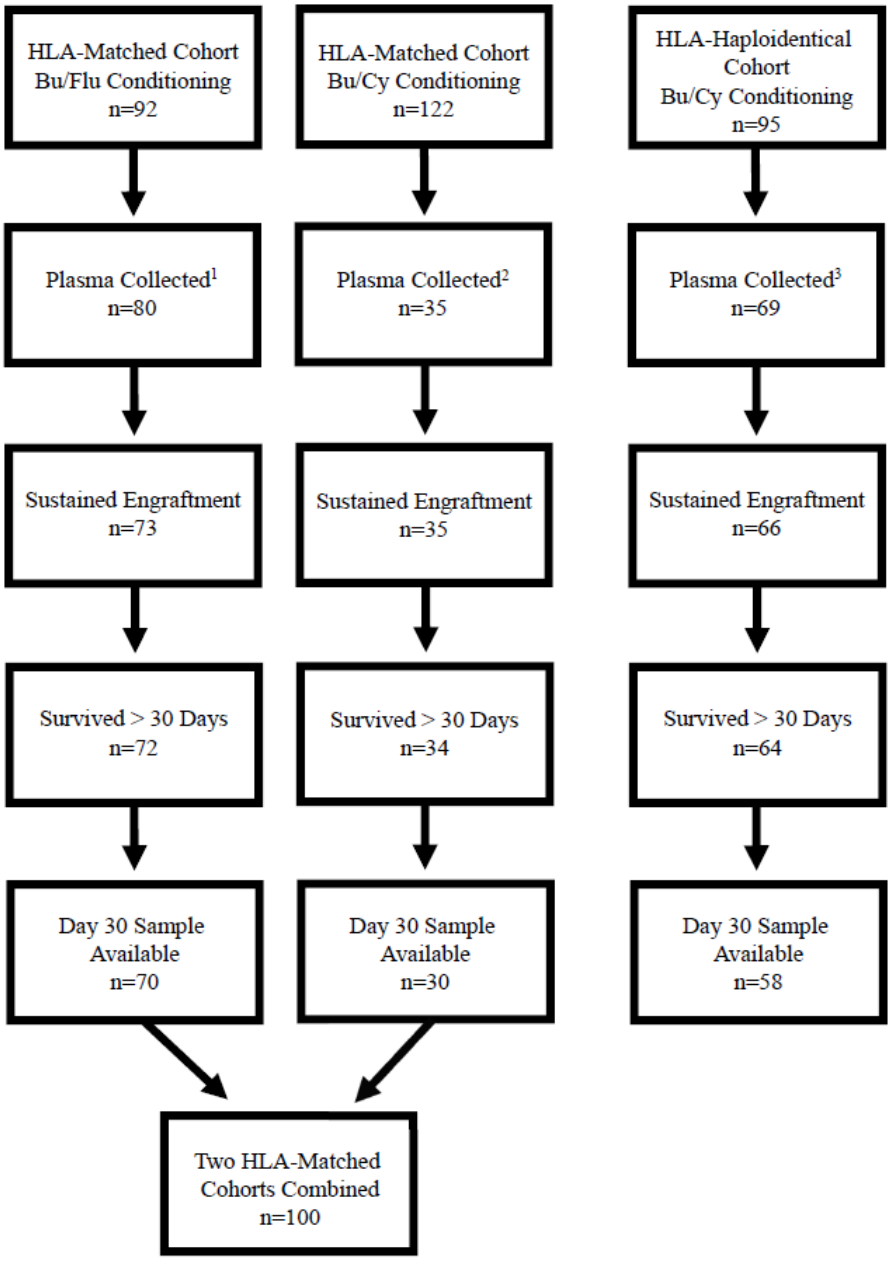

Figure S1. Flowchart showing the number of patients in each cohort. For each of the three prospective clinical studies, the total number of patients in each study, number of plasma samples collected, and number of plasma samples included are shown. The two HLA-matched cohorts, which differed only in the conditioning regimen [busulfan and cyclophosphamide (Bu/Cy) versus busulfan and fludarabine (Bu/Flu)], were combined for all analyses. [1]Plasma was only collected at two of the three participating institutions, which enrolled a total of 80 patients. [2]Plasma was only collected for a subset of 35 patients enrolled on the clinical study during 2007-2008. [3]Serial plasma specimens were collected for 92 patients. The study did not mandate specimen collection once a patient relapsed. Thus, the 23 patients that relapsed less than 6 months post-transplant were not included in these analyses as serial plasma samples were not consistently available.

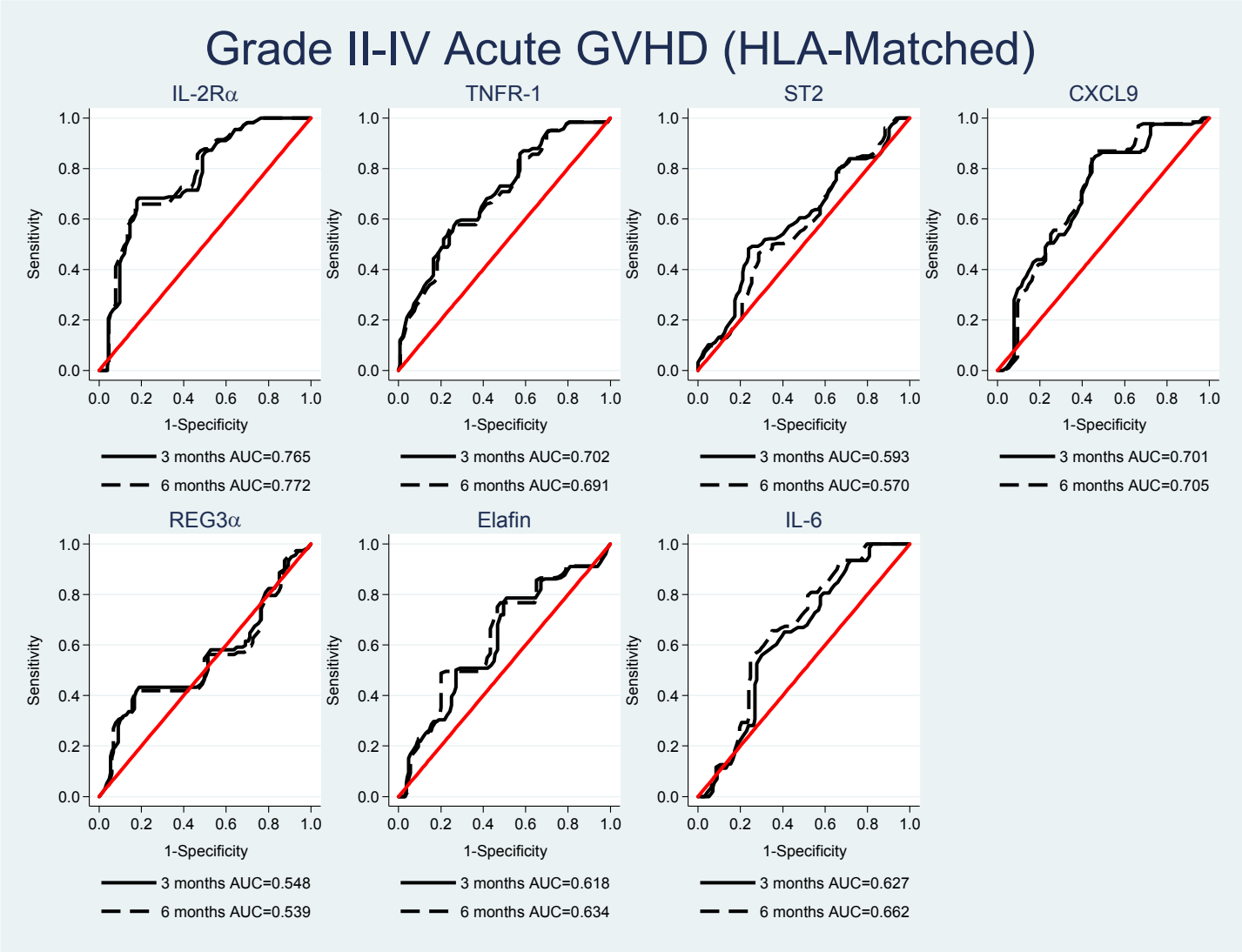

Figure S2. Time-dependent receiver operating characteristic (ROC) curves for grade II-IV acute GVHD for the HLA-matched cohort. Using day 30 biomarker levels, ROC curves for prognosticating subsequent occurrence of grade II-IV acute GVHD by 3 or 6 months post-transplant are shown for the HLA-matched cohort. The analysis was performed using a competing risk framework with non-relapse mortality, relapse, donor lymphocyte infusion, and chronic GVHD as competing risks for acute GVHD. Patients (n=26, 26%) experiencing onset of grade II-IV acute GVHD prior to day 30 were excluded from these analyses.

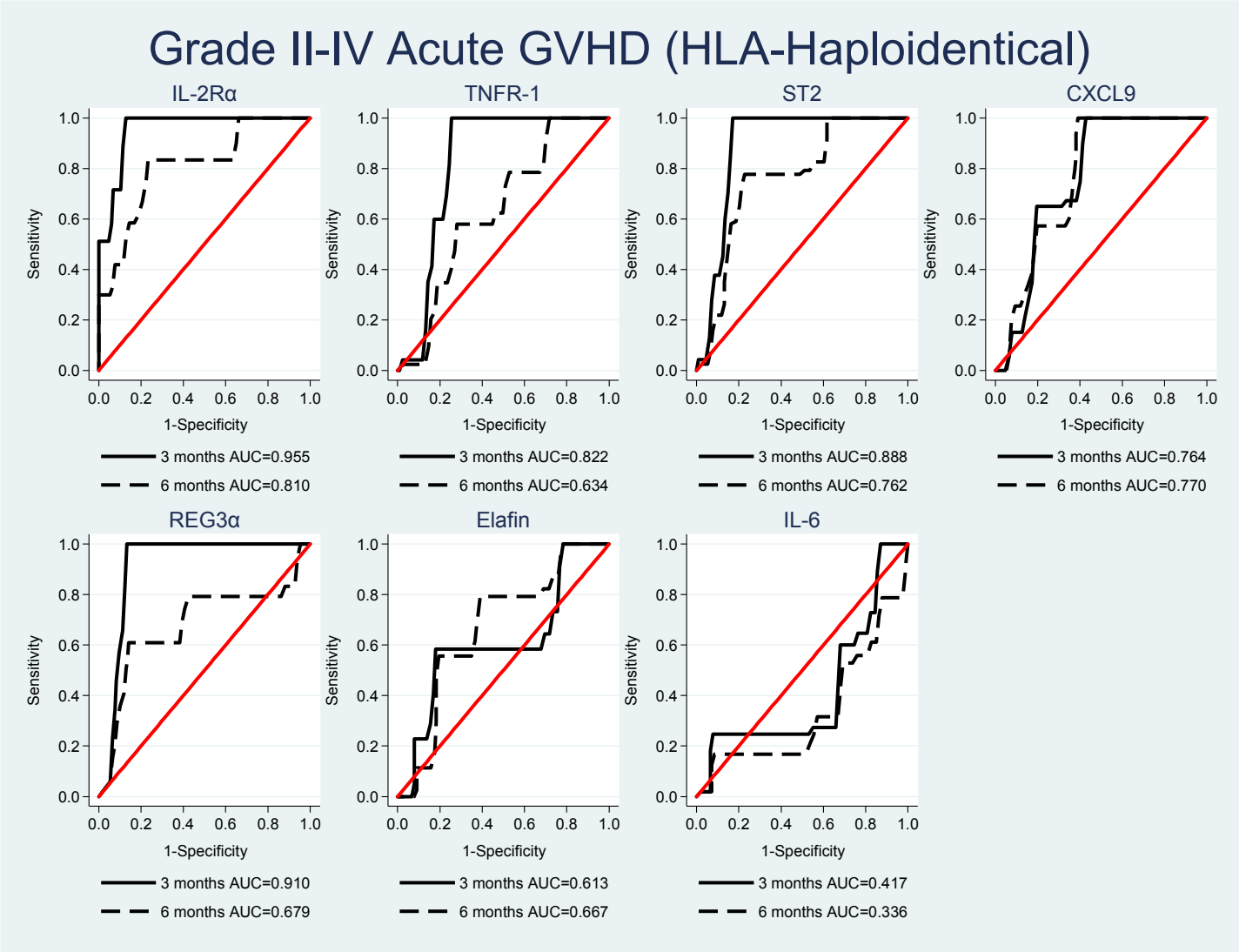

Figure S3. Time-dependent ROC curves for grade II-IV acute GVHD for the HLA-haploidentical cohort. Using day 30 biomarker levels, ROC curves for prognosticating subsequent occurrence of grade II-IV acute GVHD by 3 or 6 months post-transplant are shown for the HLA-haploidentical cohort. The analysis was performed using a competing risk framework with non-relapse mortality, relapse, donor lymphocyte infusion, and chronic GVHD as competing risks for acute GVHD. Patients (n=5, 8.6%) experiencing onset of grade II-IV acute GVHD prior to day 30 were excluded from these analyses.

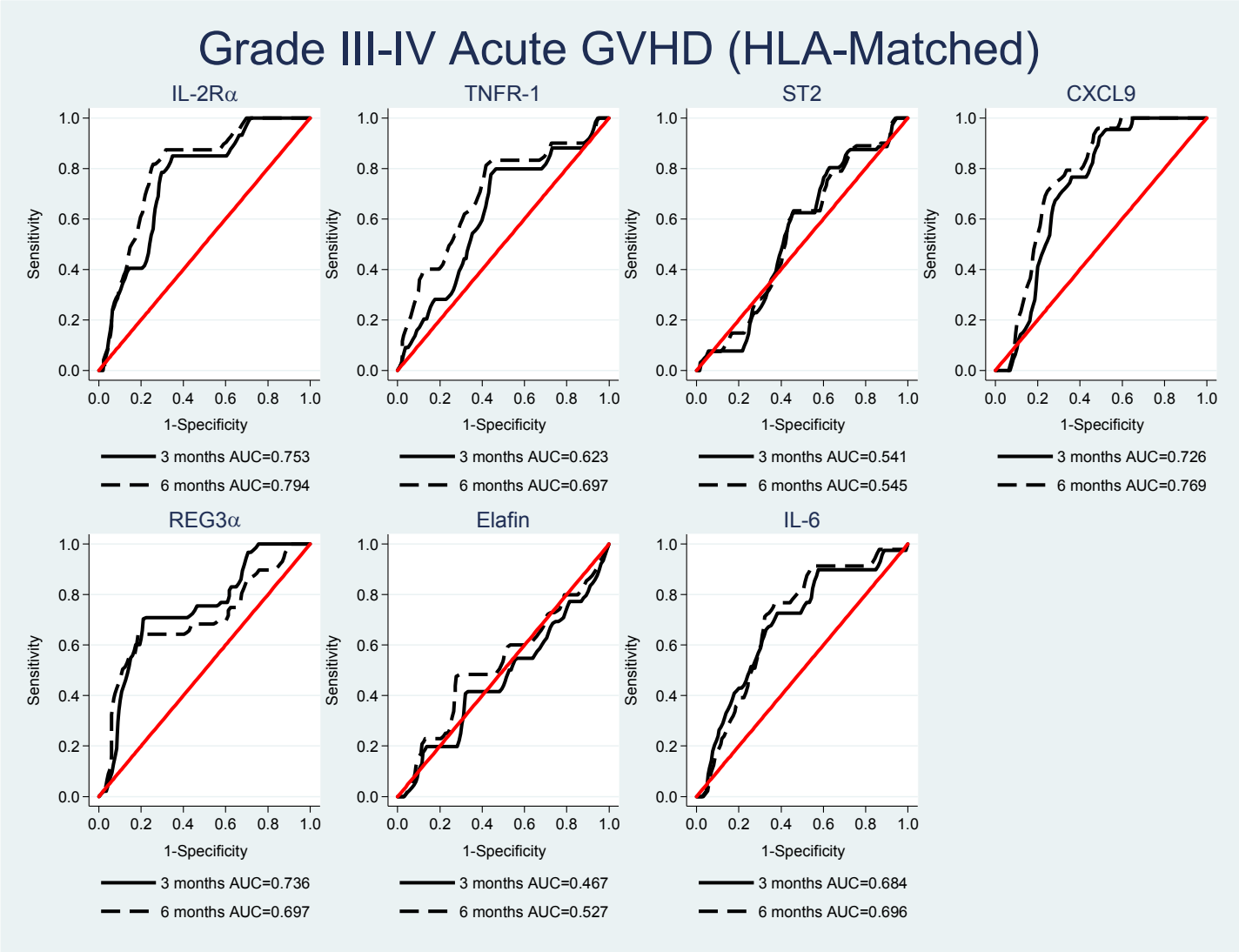

Figure S4. Time-dependent ROC curves for grade III-IV acute GVHD for the HLA-matched cohort. Using day 30 biomarker levels, ROC curves for prognosticating subsequent occurrence of grade III-IV acute GVHD by 3 or 6 months post-transplant are shown for the HLA-matched cohort. The analysis was performed using a competing risk framework with non-relapse mortality, relapse, donor lymphocyte infusion, and chronic GVHD as competing risks for acute GVHD. Patients (n=26, 26%) experiencing onset of grade II-IV acute GVHD prior to day 30 were excluded from these analyses.

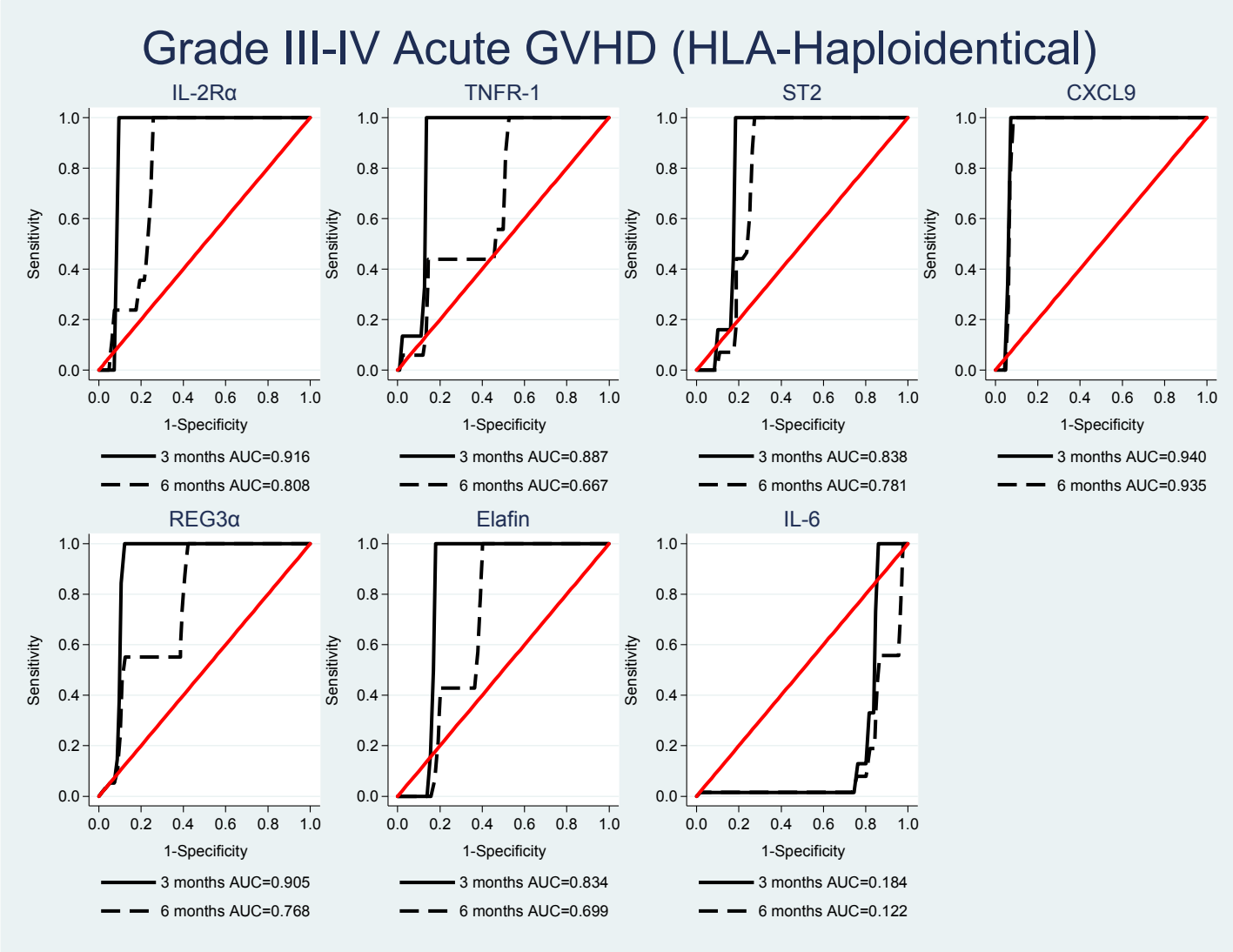

Figure S5. Time-dependent ROC curves for grade III-IV acute GVHD for the HLA-haploidentical cohort. Using day 30 biomarker levels, ROC curves for prognosticating subsequent occurrence of grade III-IV acute GVHD by 3 or 6 months post-transplant are shown for the HLA-haploidentical cohort. The analysis was performed using a competing risk framework with non-relapse mortality, relapse, donor lymphocyte infusion, and chronic GVHD as competing risks for acute GVHD. Patients (n=5, 8.6%) experiencing onset of grade II-IV acute GVHD prior to day 30 were excluded from these analyses.