Presensitization to HY antigens in female donors prior to transplant is not associated with male recipient post-transplant HY antibody development nor with clinical outcomes

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Detailed methods:

Patients and blood samples

We measured IgG against H-Y antigens in 289 female donors (age: 18-60) of high resolution 8/8 HLA-matched HCT with myeloablative conditioning facilitated by the NMDP between 1990 and 2002, and in 24 male recipients 1 year post-HCT. The data from the NMDP cohort was mainly used for the purpose of assessing the impact of donor H-Y seropositivity on cGVHD incidence and other clinical outcomes. Furthermore, we separately studied 90 adult female donors and their corresponding male recipients who underwent HCT at Stanford University Hospital between 2005 and 2012 and survived without relapse for at least 3 months post-HCT. Their plasma samples were prospectively collected 3, 6, and 9 months, 1, 1.5, 2, 2.5, and 3 years post-HCT and cryopreserved until use. The data from the Stanford cohort was mainly used for the purpose of assessing the association between presence of HY-Ab in female donors and post-HCT HY-Ab development in male recipients. This study was approved by the institutional review board of Stanford University and CIBMTR/NMDP. All patients gave written informed consents for the cryopreservation and analyses of blood samples in accordance with the Declaration of Helsinki.

Proteomic microarrays for the detection of HY antibodies

In this study, a panel of five H-Y antigens was tested on our proteomic micrroarray:[12] DBY (DEAD box 3 peptide, Y-linked, DDX3Y), UTY (ubiquitously transcribed tetratricopeptide repeat containing, Y-linked), ZFY (zinc finger protein, Y-linked), RPS4Y (ribosomal protein S4, Y-linked), and EIF1AY (eukaryotic translation initiation factor 1A, Y-linked). Since the UTY protein is too large to assess individually, it was divided into 3 overlapping fragments (UTY_{1-3}) and then assessed separately. Mean fluorescence intensity (MFI) of each spot was obtained. The threshold of each HY-Ab seropositivity was determined from 60 healthy male donors, and the positive cutoff was defined as the third quartile + 2 times the interquartile range

(Q3+2*IQR). Relative HY-Ab quantification was shown as a factor of each H-Y seropositive threshold.

Definitions of categories

Early disease risk was defined as following: the 1st complete remission (CR) for acute leukemia; the 1st chronic phase for chronic myeloid leukemia (CML); the 1st CR or partial remission / response (PR) for lymphoma / myeloma; myelodysplastic syndrome (MDS) refractory anemia, refractory anemia with ring sideroblasts. Intermediate risk disease was defined as following: 2nd or higher CR/PR for acute leukemia and lymphoma; accelerated phase or 2nd chronic phase CML. Advanced disease risk was defined as following: relapse or primary induction failure for acute leukemia; blast phase for CML; MDS refractory anemia with excess blasts (RAEB). Disease with unknown number of CR/PR was considered as "Intermediate". Unknown status was defined as "Other". Donor types were classified into 4 groups: HLA-matched related donor (MRD), HLA-mismatched related donor (MMRD), 8/8 HLA-matched unrelated donor (MUD), and HLA-mismatched unrelated donor (MMUD). Cord blood transplant was not included in this study. The diagnosis and severity of acute GVHD (aGVHD) for both cohorts were reported based on traditional grading scores.[13] The diagnosis of chronic GVHD (cGVHD) was reported based on the classical criteria[14] and NIH classification[15] for the NMDP cohort and the Stanford cohort, respectively. To address impact of detection of multiple HY-Abs, HY-score was defined as the sum total of HY-Ab seropositivity. Regarding UTY, samples reactive with any one of UTY_{1-3} were scored as positive for UTY. HY-score indicates recognition of 0 to 5 HY-Abs.

Statistical analysis

Chi-Square or Mann-Whitney tests were used to compare discrete or continuous factors between the H-Y seropositive and seronegative female donors. Primary clinical endpoint was cGVHD incidence according to donor H-Y seropositivity. The cumulative incidences of

clinical outcomes (acute and chronic GVHD, relapse, non-relapse mortality (NRM)) were estimated by Gray's method considering death without event as a competing risk. Overall survival (OS) from HCT was estimated by the Kaplan-Meier Method and compared by log-rank test. These probabilities were estimated with a 95% confidence interval (CI). In a multivariate analysis, we examined the association of between HY-score and various outcomes of interest adjusting for potential confounders. To this end, the Cox regression was used and the primary independent variable, "HY-score in donor", was held in all models regardless of its significance level. To adjust for confounding effect, we have considered the following prognostic factors: age of patients and donors, disease, disease status, performance status, graft type, CMV serostatus, number of donor pregnancies, conditioning regimen, and GVHD prophylaxis. For each outcome of interest (e.g., time to relapse or time to cGVHD), the stepwise model selection procedures were applied to select confounding factors along with HY-score in donor to build a prognostic model with a threshold P-value of 0.05 for variable selections. Due to the stepwise variable selection, the nominal p-value and confidence intervals of the coefficients for the selected variables may not be reliable and further validation with independent data is needed. In all the final models, the proportional hazard assumption for all prognostic variables under consideration was examined by testing their interaction with a transformation of time. In all the analyses, P-value < 0.05 was considered to be significant except for the analysis of individual HY-Abs, where P<0.01 was applied for the purpose of addressing the potential concern of multiple comparisons. SAS software version 9.2 (SAS Institute, Cary, NC) was used for the analysis of the NMDP cohort, and Stata ver.12.0 (StataCorp, College Station, TX, USA) and EZR (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html)[16] were used for the analysis of the Stanford cohort.

Table S1. Patient characteristics

	NMDP	Stanford
Number of patients	289	90
Year of transplant	1990-2002	2005-2012
Age, median (range), years	34 (<1-59)	52 (21-74)
Donor age, median (range), years	37 (18-60)	47 (20-73)
Graft Type		
Bone Marrow	270 (93%)	3 (3)
Peripheral blood	19 (7%)	87 (97%)
Disease at transplant		
AML	53 (18%)	24 (27%)
ALL	70 (24%)	15 (17%)
Other leukemia	5 (2%)	7 (8%)
CML	109 (38%)	3 (3%)
MDS	38 (13%)	9 (10%)
Non-Hodgkin Lymphoma	13 (4%)	28 (31%)
Others	1 (<1%)	4 (4%)
Donor types		
MRD	0	68 (76%)
MUD	289 (100%)	15 (17%)
MMRD	0	2 (2%)
MMUD	0	5 (6%)
Disease status at transplant		
Early	110 (38%)	36 (40%)
Intermediate	108 (37%)	34 (38%)
Advanced	38 (13%)	12 (13%)
Other	33 (11%)	8 (9%)
Donor/recipient CMV match		
Negative/Negative	102 (35%)	19 (21%)
Negative/Positive	68 (24%)	15 (17%)
Positive/Negative	60 (21%)	17 (19%)
Positive/Positive	54 (19%)	39 (43%)
Unknown	5 (1%)	0
Conditioning regimen		
MAC	289 (100%)	39 (43%)
RIC	0	51 (57%)
GVHD prophylaxis		
CSA / TAC + MTX	226 (78%)	17 (19%)
CSA / TAC + MMF	0	53 (59%)
Others	0	20 (22%)
T cell depletion	63 (22%)	0

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; MRD, HLA matched related donor; MUD, HLA matched unrelated donor; MMRD, HLA mismatched related donor; MMUD, HLA mismatched unrelated donor; CMV, cytomegalovirus; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus; MTX, methotrexate; MMF, mycophenolate mofetil.

Table S2. Association between the detection of individual HY antibodies and donor age or donor pregnant history in the NMDP cohort

	Any HY-Abs					DBY		UTY		RPS4Y		ZFY		EIF1AY	
Number of HY-Abs	0	>=1	P	>=3	P	Positive	P	Positive	P	Positive	P	Positive	P	Positive	P
Total (n=289)	146	143		29		63		112		42		22		7	
Median of donor age (range)	36 (19-59)	37 (18-56)	0.30	40 (23-56)	0.02	36 (18-56)	0.86	37 (18-56)	0.31	38 (18-56)	0.54	40 (23-50)	0.06	45 (24-50)	0.07
Donor parity															
0 (n=110)	56 (38%)	54 (38%)	0.88	12 (41%)	0.24	28 (45%)	0.24	38 (35%)	0.34	19 (46%)	0.54	6 (86%)	0.87	9 (41%)	0.06
1 (n=47)	22 (15%)	25 (17%)		7 (24%)		12 (19%)		22 (20%)		5 (12%)		0		4 (18%)	
>2 (n=127)	65 (45%)	62 (43%)		9 (31%)		22 (35%)		50 (45%)		17 (40%)		1 (14%)		9 (41%)	

Table S3. Number of multiple HY antibody recognition (HY-score) and clinical outcomes in the NMDP cohort.

		cGVHD		grade II-IV aGVHD		Relapse		NRM		OS	
		HR	P	HR	P	HR	P	HR	P	HR	P
HY-score	0 (n=146)	1	-	1	-	1		1	-	1	-
	1 (n=75)	1.34 (0.90-1.99)	0.15	1.17 (0.78-1.75)	0.46	1.22 (0.65-2.27)	0.54	0.85 (0.56-1.29)	0.44	0.98 (0.69-1.39)	0.89
	2 (n=39)	1.12 (0.69-1.81)	0.65	1.25 (0.76-2.05)	0.39	0.63 (0.26-1.53)	0.31	0.62 (0.36-1.06)	0.078	0.81 (0.50-1.31)	0.39
	3 or more (n=29)	1.32 (0.72-2.43)	0.37	1.64 (0.97-2.75)	0.064	1.85 (0.74-4.62)	0.19	1.30 (0.76-2.24)	0.34	1.48 (0.93-2.37)	0.099
Patient age	0 - 9	1		1		-		1		1	
	10 -19	2.50 (1.06-5.90)	0.036	1.39 (0.60-3.22)	0.44	-		5.42 (2.08-14.1)	0.0005	3.04 (1.56-5.90)	0.001
	20 -29	3.12 (1.56-6.23)	0.0013	1.86 (0.92-3.76)	0.085	-		3.19 (1.32-7.74)	0.01	2.12 (1.17-3.84)	0.014
	30 -39	3.06 (1.53-6.12)	0.0016	1.79 (0.89-3.60)	0.1	-		4.75 (1.98-11.4)	0.0005	3.10 (1.68-5.72)	0.0003
	40 -49	4.27 (2.15-8.47)	<0.0001	2.97 (1.52-5.79)	0.0014	-		4.77 (1.98-11.5)	0.0005	2.61 (1.42-4.78)	0.002
	50 -	4.12 (1.94-8.75)	0.0002	2.63 (1.24-5.61)	0.012	-		5.56 (2.21-14.0)	0.0003	2.79 (1.39-5.61)	0.0039
Disease	AML	-		-		-		-		1	
	ALL	-		-		-		-		1.04 (0.66-1.63)	0.88
	NHL	-		-		-		-		2.61 (1.34-5.10)	0.0049
	CML	-		-		-		-		0.65 (0.43-0.98)	0.039
	MDS	-		-		-		-		0.60 (0.35-1.02)	0.059
Disease Status	Early	-		-		1		-		-	
	Intermediate	-		-		3.11 (1.43-6.75)	0.0042	-		-	
	Advanced	-		-		5.95 (2.47-14.4)	0.0001	-		-	
	Other	-		-		4.80 (1.46-15.7)	0.0096	-		-	
	missing	-		-		5.39 (1.76-16.5)	0.0033	-		-	
KPS	10-80	-		-		1		-		1	
	90-100	-		-		0.43 (0.24-0.76)	0.0036	-		0.58 (0.42-0.79)	0.0006
Donor Parity	0	-		-		-		1		-	
	1	-		-		-		1.34 (0.80-2.24)	0.27	-	
	2	-		-		-		2.03 (1.29-3.19)	0.0021	-	
	3	-		-		-		1.30 (0.77-2.21)	0.33	-	
	4 or more	-		-		-		1.06 (0.59-1.88)	0.86	-	
Conditioning	TBI-CY	-		-		-		1		-	
	TBI-other	-		-		-		3.25 (1.53-6.92)	0.0022	-	
	Busulfan-based	-		-		-		1.47 (0.92-2.34)	0.11	-	

cGVHD, chronic graft-versus-host disease: aGVHD, acute graft-versus-host disease; NRM, non-relapse mortality; OS, overall survival; HR, hazard ratio; CI, confidence interval; P, P-value; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; KPS, Karnofsky performance status; TBI, total body irradiation. “-” indicates that the factor did not remain significant through stepwise deletion approach in multivariate analyses. Due to the stepwise variable selection, the nominal p-value and confidence intervals of selected prognostic factors other than HY-score may not be valid and need to be interpreted with caution.

Figure S1. Other clinical outcomes in male recipients according to the detection of multiple HY-antibodies in female donors (donor HY-score) prior to transplant

Female donor HY-score prior to transplant was not associated with other clinical outcomes. (Figures are for the NMDP cohort)

Figure S1
Donor HY-Score was not associated with other clinical outcomes.