SUPPLEMENTARY APPENDIX

Risks and benefits of sex-mismatched hematopoietic cell transplantation differ according to conditioning strategy

Hideki Nakasone,[1,2] Mats Remberger,[3] Lu Tian,[4] Petter Brodin,[3,5] Bita Sahaf,[1] Fang Wu,[1] Jonas Mattsson,[3] Robert Lowsky,[1] Robert Negrin,[1] David B.Miklos,[1*] and Everett Meyer[1*]

[1]Division of Blood and Marrow Transplantation, Stanford University School of Medicine, Stanford, CA, USA; [2]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [3]Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; [4]Department of Health Research and Policy, Stanford University School of Medicine, CA, USA; [5]Science for Life Laboratory, Department of Medicine, Karolinska Institutet, Stockholm, Sweden

DBM and EM contributed equally this work.

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Correspondence: evmeyer@stanford.edu

Supplementary methods

- **Definitions of categories**

Conditioning regimens were classified into 3 groups: TLI-ATG, RIC, and MAC. The TLI-ATG conditioning protocol (n=430) was reported previously.[1-3] Briefly, TLI was administered at a dose of 0.8 Gy/day for a total dose of 8 Gy. ATG was infused intravenously at 1.5 mg/kg/day x 5 days. RIC (n=266) mainly included fludarabine (Flu)-based conditioning regimens: Flu /busulfan (Bu) in 103, Flu /cyclophosphamide (CY) /±total body irradiation (TBI)/±α in 62, Flu /carmustine /melpharan /±α in 53, Flu/TBI in 11, and others in 37. MAC (n=345) included Bu/CY in 140, TBI/CY in 71, TBI /etoposide (ETP) /±CY in 61, Bu /ETP /CY in 39, carmustine /ETP /CY in 32, and others in 2.

Donor types were classified into 3 groups: HLA-matched related donor (MRD), 8/8 HLA-matched unrelated donor (MUD), and HLA-mismatched unrelated donor (MMUD). Cord blood transplant was not included in this study. Standard risk disease was defined as following: the 1st complete remission in AML; the 1st chronic phase in chronic myeloid leukemia; myelodysplastic syndrome (MDS) other than refractory anemia with excess blasts; complete remission in CLL, lymphoma, and plasma cell disorder. Other disease status or unknown status was defined as high risk disease. The diagnosis and severity of aGVHD were reported based on traditional grading scores,[4] while those of cGVHD were determined based on the NIH classification.[5]

- **Detection of HY antibodies in Female→Male HCT**

Antibodies against 5 HY- antigens were measured in a proteomic microarray, using plasma samples diluted at 1:50:[6] DBY (DEAD box 3 peptide, Y-linked, DDX3Y), UTY (ubiquitously transcribed tetratricopeptide repeat containing, Y-linked), ZFY (zinc finger protein, Y-linked), EIF1AY (eukaryotic translation initiation factor 1A, Y-linked) , RPS4Y (ribosomal protein S4, Y-linked). Mean fluorescence intensity (MFI) of each spot was

obtained. Since UTY are too large to assess, it was divided into 3 overlapped fragments (UTY_{1-3}) and assessed separately. Samples reactive with any one of UTY_{1-3} were scored as positive for UTY. The threshold of each HY-antibody seropositivity was determined from 60 healthy male donors, and the positive cutoff was defined as the third quartile + 2 times of interquartile range (Q3+2*IQR).

- **Statistical analysis**

Chi-square test was performed for categorical variables. The cumulative probabilities of aGVHD and cGVHD were estimated by Gray's method, considering relapse and death without GVHD as competing risks. The analyses of cGVHD included only patients who survived without relapse at least 100 days post-HCT. The cumulative probabilities of relapse and non-relapse mortality were also estimated by Gray's method, considering each other as a competing risk. Overall survival (OS) from HCT was estimated by the Kaplan-Meier Method and compared by log-rank test. Since our primary aim was to address the difference of impact of sex-mismatch among different conditioning regimens, we assessed effects of sex-mismatched HCT in the TLI-ATG, RIC, and MAC groups, separately. Multivariate analyses were performed using Cox proportional hazard model, and hazard ratio (HR) of sex-mismatch or detection of HY-antibody was adjusted for patient age, disease, disease risk, patient cytomegalovirus (CMV) seropositivity, and donor types (MRD vs. MUD vs. MMUD).[7,8] Probabilities and HR(s) were estimated with a 95% confidence interval (CI). Since OS of sex-matched HCT was not significantly different between Female→Female and Male→Male HCT in overall cohort, we consider both of F→F and M→M HCT as one group of sex-matched HCT. Two-tailed P-value < 0.05 was considered significant. All analyses and data management were performed using Stata ver.12.0 (StataCorp, College Station, TX, USA), and EZR[9] (Saitama Medical Center, Jichi Medical University at http://www.jichi.ac.jp/saitama-sct/ SaitamaHP.files/statmedEN.html).[9]

References:

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8. Kanda J, Nakasone H, Atsuta Y, et al. Risk factors and organ involvement of chronic GVHD in Japan. Bone Marrow Transplant. 2014;49(2):228-235.
9. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48(3):452-458.

Table S1 Patient characteristics

	MAC (n=345)		RIC (n=266）		TLIATG (n=430)		P-value
Age	n	%	n	%	n	%	
18-35	73	21%	15	6%	28	7%	<0.001
35-50	165	48%	45	17%	46	11%	
50-	107	31%	206	77%	356	83%	
Gender							
Female	163	47%	109	41%	175	41%	0.14
Male	182	53%	157	59%	255	59%	
Disease							
AML	195	57%	94	35%	126	29%	<0.001
Lymphoma MM	58	17%	103	39%	224	52%	
MDS MPN	92	27%	69	26%	80	19%	
Disease risk							
standard	159	46%	93	35%	247	57%	<0.001
high	186	54%	173	65%	183	43%	
Recipient CMV							
negative	102	30%	70	26%	197	46%	<0.001
positive	243	70%	196	74%	233	54%	
Donor CMV							
negative	162	47%	115	43%	216	50%	0.21
positive	182	53%	150	56%	214	50%	
Donor							
MRD	194	56%	99	37%	197	46%	<0.001
MUD	122	35%	150	56%	166	39%	
MMUD	29	8%	17	6%	67	16%	
Sex mismatch							
Match	188	54%	186	70%	219	51%	<0.001
Female to male	68	20%	42	16%	114	27%	
Male to female	89	26%	38	14%	97	23%	
ABO mismatch							
Match	175	51%	129	48%	224	52%	0.14
Major	74	21%	63	24%	89	21%	
Minor	79	23%	47	18%	87	20%	
Major-Minor	16	5%	27	10%	30	7%	
GVHD prophylaxis							
CsA_based	99	29%	200	75%	430	100%	<0.001
FK-based	246	71%	66	25%	0	0%	
with ATG	69	20%	170	64%	430	100%	<0.001
without ATG	276	80%	96	36%	0	0%	

TLI-ATG, total lymphocyte irradiation with anti-thymocyte globulin; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; AML, acute myelogenous leukemia; MDS, myelodysplasitc syndrome; MPN, myeloproliferative neoplasm; CMV, cytomegalovirus; MRD, HLA-matched related donor; MUD, HLA-matched unrelated donors; MMUD, HLA-mismatched unrelated donors; CsA, cyclosporine; Tac, tacrolimus; ATG, anti-thymocyte globulin (including alemtuzumab

Table S2. Multivariate analyses for the impact of conditioning type on survival in overall cohort and the subgroups stratified by sex-mismatch

	Overall cohort		F→M HCT		M→F HCT		Sex-matched HCT	
	Hazard ratio (95%CI)	p.value	Hazard ratio (95%CI)	p.value	Hazard ratio (95%CI)	p.value	Hazard ratio (95%CI)	p.value
Conditioning type								
TLIATG	0.95 (0.63-1.42)	0.79	0.37 (0.15-0.93)	0.035	1.31 (0.48-3.60)	0.60	1.15 (0.69-1.92)	0.60
RIC	0.72 (0.52-0.99)	0.045	0.46 (0.22-0.95)	0.036	1.07 (0.49-2.36)	0.86	0.75 (0.49-1.12)	0.16
MAC	1	-	1	-	1	-	1	-
Age	1.02 (1.01-1.03)	<0.001	1.02 (1.00-1.04)	0.069	1.00 (0.98-1.03)	0.80	1.02 (1.01-1.04)	<0.001
CMV seropositivity	0.95 (0.77-1.16)	0.59	0.82 (0.55-1.22)	0.33	1.30 (0.79-2.15)	0.31	0.97 (0.74-1.27)	0.81
Disease								
AML	1	-	1	-	1	-	1	-
Lymphoma	0.62 (0.49-0.80)	<0.001	0.90 (0.56-1.46)	0.67	0.35 (0.18-0.68)	0.002	0.63 (0.46-0.88)	0.006
MDS	0.96 (0.75-1.22)	0.74	1.26 (0.74-2.14)	0.40	1.08 (0.63-1.84)	0.78	0.88 (0.64-1.21)	0.430
High Risk disease	1.28 (1.05-1.57)	0.016	1.61 (1.06-2.44)	0.02	1.46 (0.91-2.35)	0.12	1.15 (0.88-1.50)	0.316
Donor type								
MRD	1	-	1	-	1	-	1	-
MUD	1.02 (0.80-1.29)	0.88	0.71 (0.44-1.14)	0.15	1.32 (0.80-2.18)	0.28	1.02 (0.73-1.42)	0.89
MMUD	1.08 (0.78-1.50)	0.64	0.66 (0.35-1.26)	0.21	1.21 (0.50-2.91)	0.68	1.20 (0.77-1.85)	0.42
ATG usage	0.82 (0.58-1.16)	0.25	1.04 (0.47-2.33)	0.92	0.79 (0.34-1.81)	0.58	0.84 (0.53-1.33)	0.47
Sex-mismatch								
Match	1	-	-		-		-	
Female to male	1.08 (0.85-1.37)	0.53	-		-		-	
Male to female	0.81 (0.62-1.04)	0.097	-		-		-	

TLI-ATG, totoal lymphocyte irradiation with anti-thymocyte globulin; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; AML, acute myelogenous leukemia; MDS, myelodysplasitc syndrome; MPN, myeloproliferative neoplasm; CMV, cytomegalovirus; MRD, HLA-matched related donor; MUD, HLA-matched unrelated donors; MMUD, HLA-mismatched unrelated donors, ATG, anti-thymocyte globulin (including alemtuzumab)

Supplementary figure legends:

Figure S1. Impacts of sex-mismatched transplant within each gender on clinical outcomes. Each hazard ratio is shown after adjusting for patient age, disease, disease risk, patient cytomegalovirus seropositivity, and donor types. TLI-ATG, total lymphoid irradiation with anti-thymocyte globulin; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; HR, hazard ratio; CI, confidence interval. *Patients with myelodysplastic syndrome and chronic lymphocytic leukemia were excluded.

Figure S2. Relapse incidence according to sex-mismatch in the total lymphoid irradiation with anti-thymocyte globulin (TLI-ATG) group differs by disease types.
Graft-versus-leukemia/lymphoma effect by sex-mismatch was prominent in acute myelogenous leukemia (AML, upper left) and non-Hodgkin lymphoma (NHL, upper right) other than chronic lymphoid leukemia (CLL), but not observed in patients with myelodysplastic syndrome (MDS, lower left) or CLL (lower right).

Figure S3. (A) Disease relapse and (B) chronic GVHD incidence according to the detection of HY antibodies 3m post-F→M HCT with myeloablative conditioning

Figure S4. Overall survival according to HLA mismatch among HCT from unrelated donors (A) in patients with total lymphoid irradiation with anti-thymocyte globulin (TLI-ATG) and (B) in patients (non-TLI-ATG group) with other reduced-intensity conditioning (RIC) and myeloablative conditioning (MAC)

Figure S5. Overall survival according to conditioning strategy in overall cohort and subgroups stratified by sex-mismatch

Figure S6. Overall survival according to sex-mismatch in myeloablative (MAC) and reduced-intensity conditioning (RIC) patients with and without ATG

Figure S1.

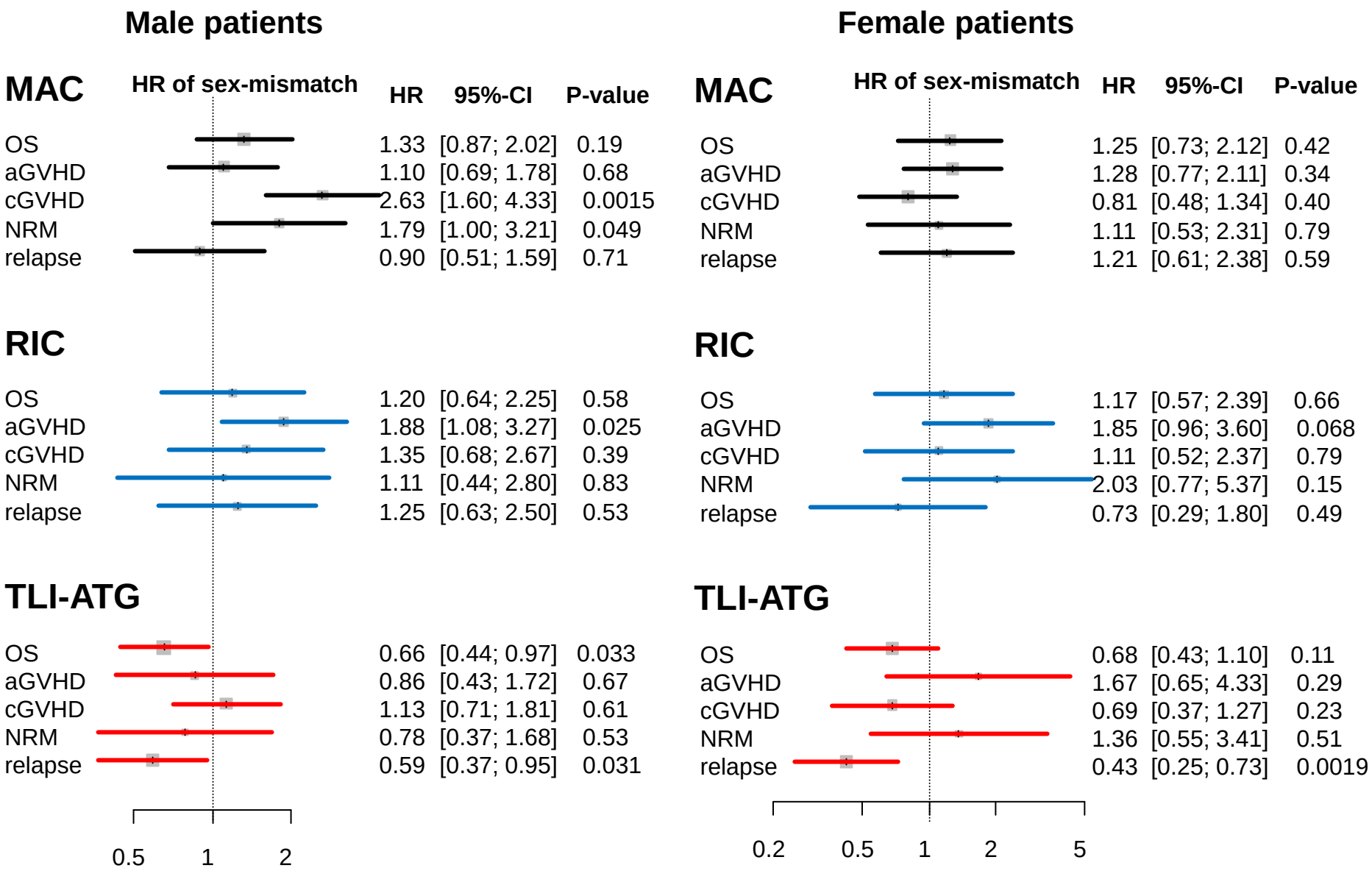

Male patients
Female patients
MAC
HR of sex-mismatch
HR
95%-CI
P-value
OS 1.33 [0.87; 2.02] 0.19
aGVHD 1.10 [0.69; 1.78] 0.68
cGVHD 2.63 [1.60; 4.33] 0.0015
NRM 1.79 [1.00; 3.21] 0.049
relapse 0.90 [0.51; 1.59] 0.71
RIC
OS 1.20 [0.64; 2.25] 0.58
aGVHD 1.88 [1.08; 3.27] 0.025
cGVHD 1.35 [0.68; 2.67] 0.39
NRM 1.11 [0.44; 2.80] 0.83
relapse 1.25 [0.63; 2.50] 0.53
TLI-ATG
OS 0.66 [0.44; 0.97] 0.033
aGVHD 0.86 [0.43; 1.72] 0.67
cGVHD 1.13 [0.71; 1.81] 0.61
NRM 0.78 [0.37; 1.68] 0.53
relapse 0.59 [0.37; 0.95] 0.031
0.5
1
2
MAC
HR of sex-mismatch
HR
95%-CI
P-value
OS 1.25 [0.73; 2.12] 0.42
aGVHD 1.28 [0.77; 2.11] 0.34
cGVHD 0.81 [0.48; 1.34] 0.40
NRM 1.11 [0.53; 2.31] 0.79
relapse 1.21 [0.61; 2.38] 0.59
RIC
OS 1.17 [0.57; 2.39] 0.66
aGVHD 1.85 [0.96; 3.60] 0.068
cGVHD 1.11 [0.52; 2.37] 0.79
NRM 2.03 [0.77; 5.37] 0.15
relapse 0.73 [0.29; 1.80] 0.49
TLI-ATG
OS 0.68 [0.43; 1.10] 0.11
aGVHD 1.67 [0.65; 4.33] 0.29
cGVHD 0.69 [0.37; 1.27] 0.23
NRM 1.36 [0.55; 3.41] 0.51
relapse 0.43 [0.25; 0.73] 0.0019
0.2
0.5
1
2
5

Figure S2.

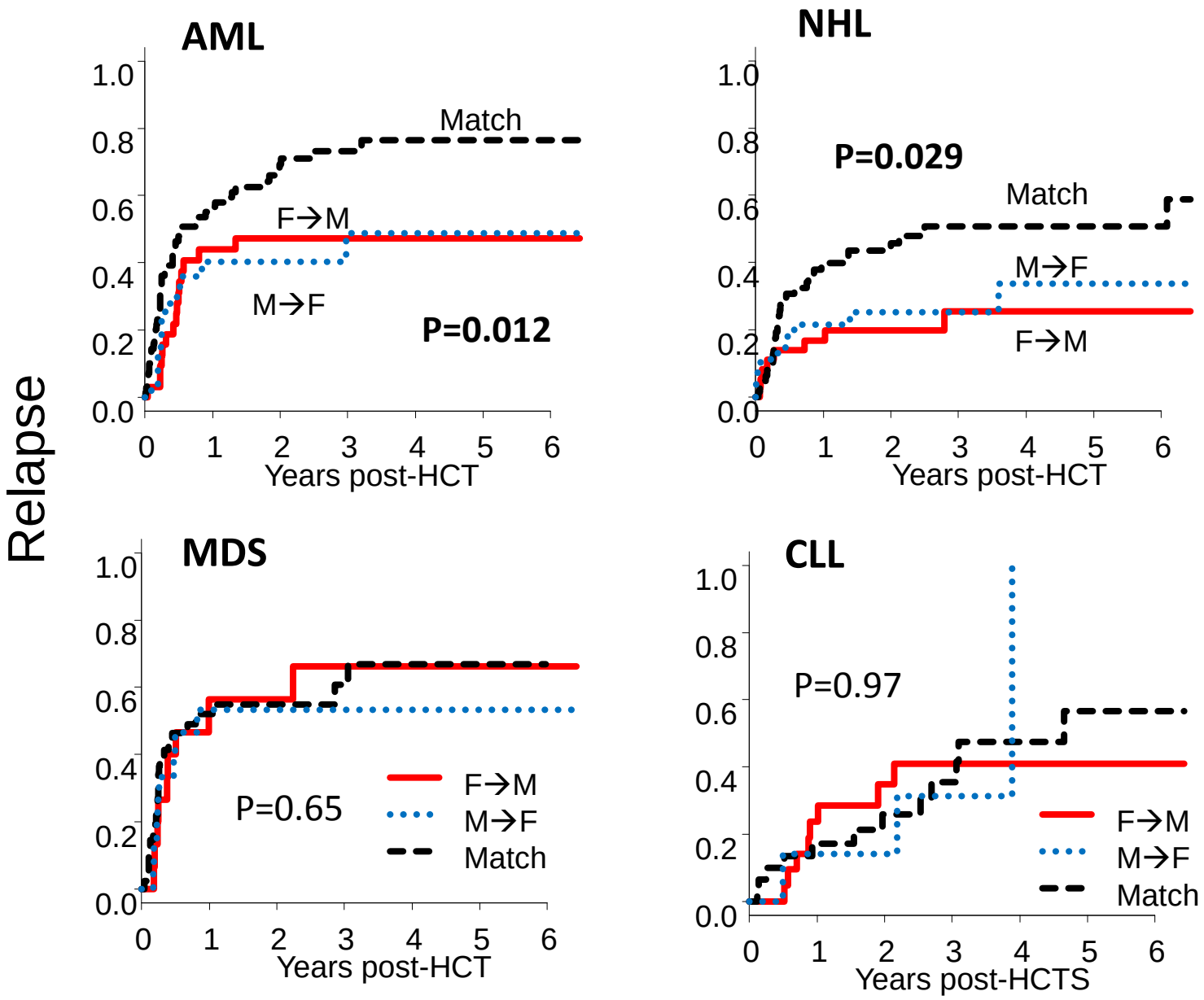
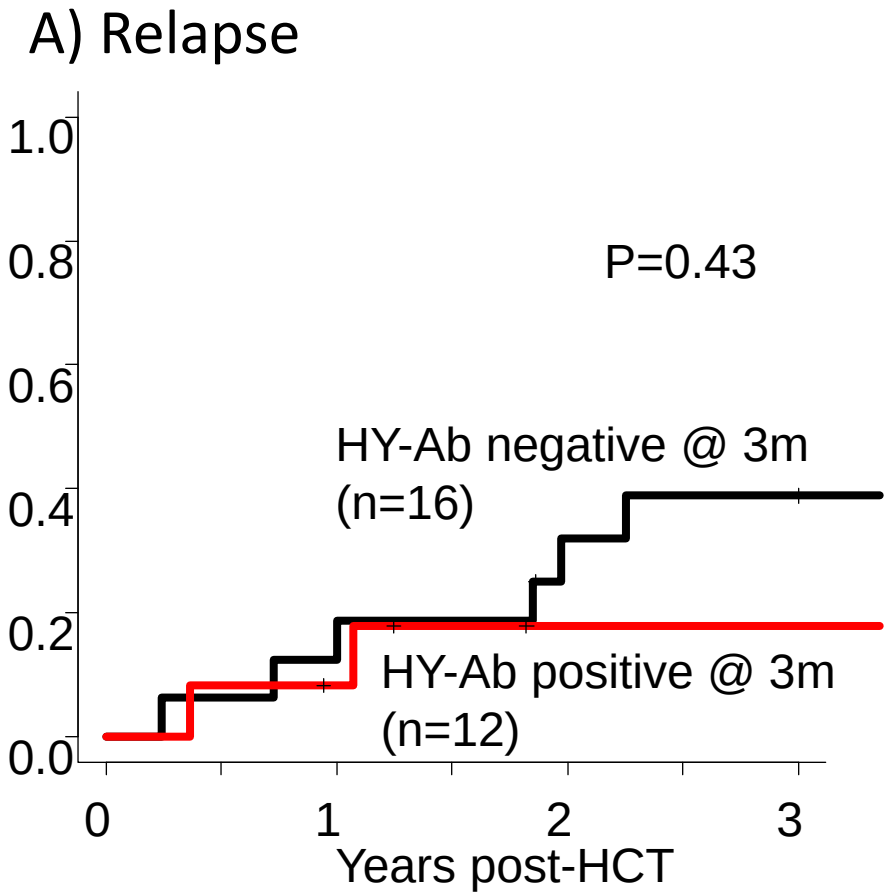

AML
Relapse
1.0
0.8
0.6
0.4
0.2
0.0
Match
F→M
M→F
P=0.012
0 1 2 3 4 5 6
Years post-HCT
NHL
P=0.029
Match
M→F
F→M
Years post-HCT
MDS
P=0.65
F→M
M→F
Match
Years post-HCT
CLL
P=0.97
F→M
M→F
Match
Years post-HCTS

Figure S3


A) Relapse
1.0
0.8
0.6
0.4
0.2
0.0
P=0.43
HY-Ab negative @ 3m
(n=16)
HY-Ab positive @ 3m
(n=12)
0
1
2
3
Years post-HCT

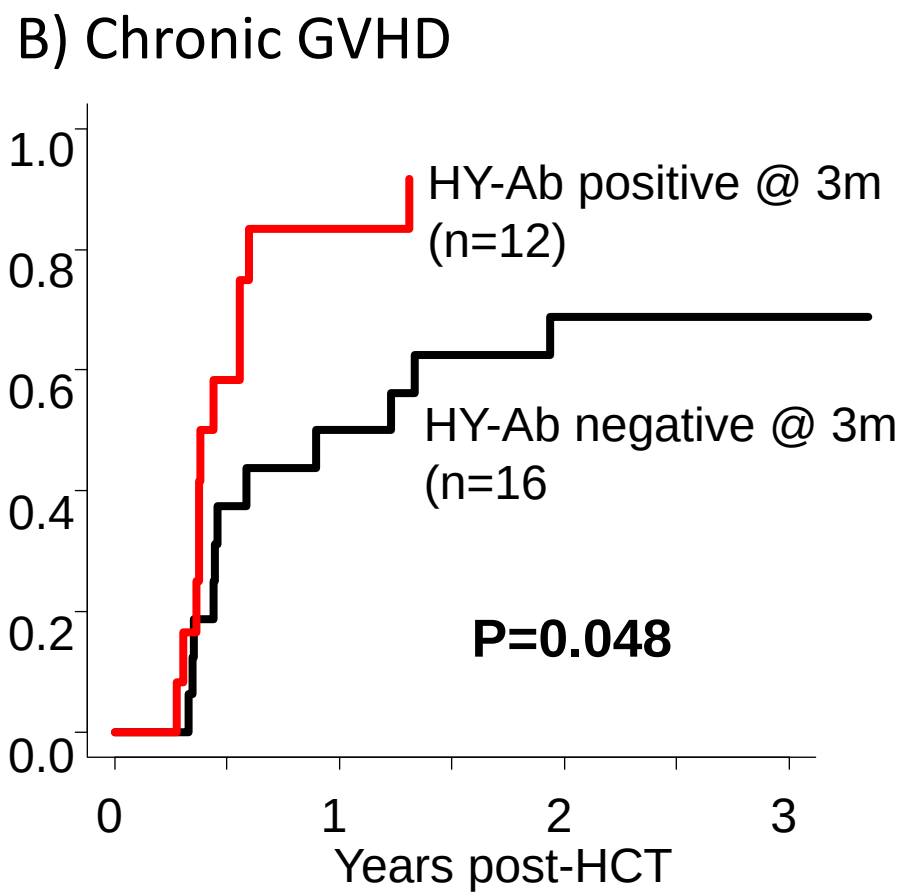

B) Chronic GVHD
1.0
0.8
0.6
0.4
0.2
0.0
HY-Ab positive @ 3m
(n=12)
HY-Ab negative @ 3m
(n=16
P=0.048
0
1
2
3
Years post-HCT

Figure S4

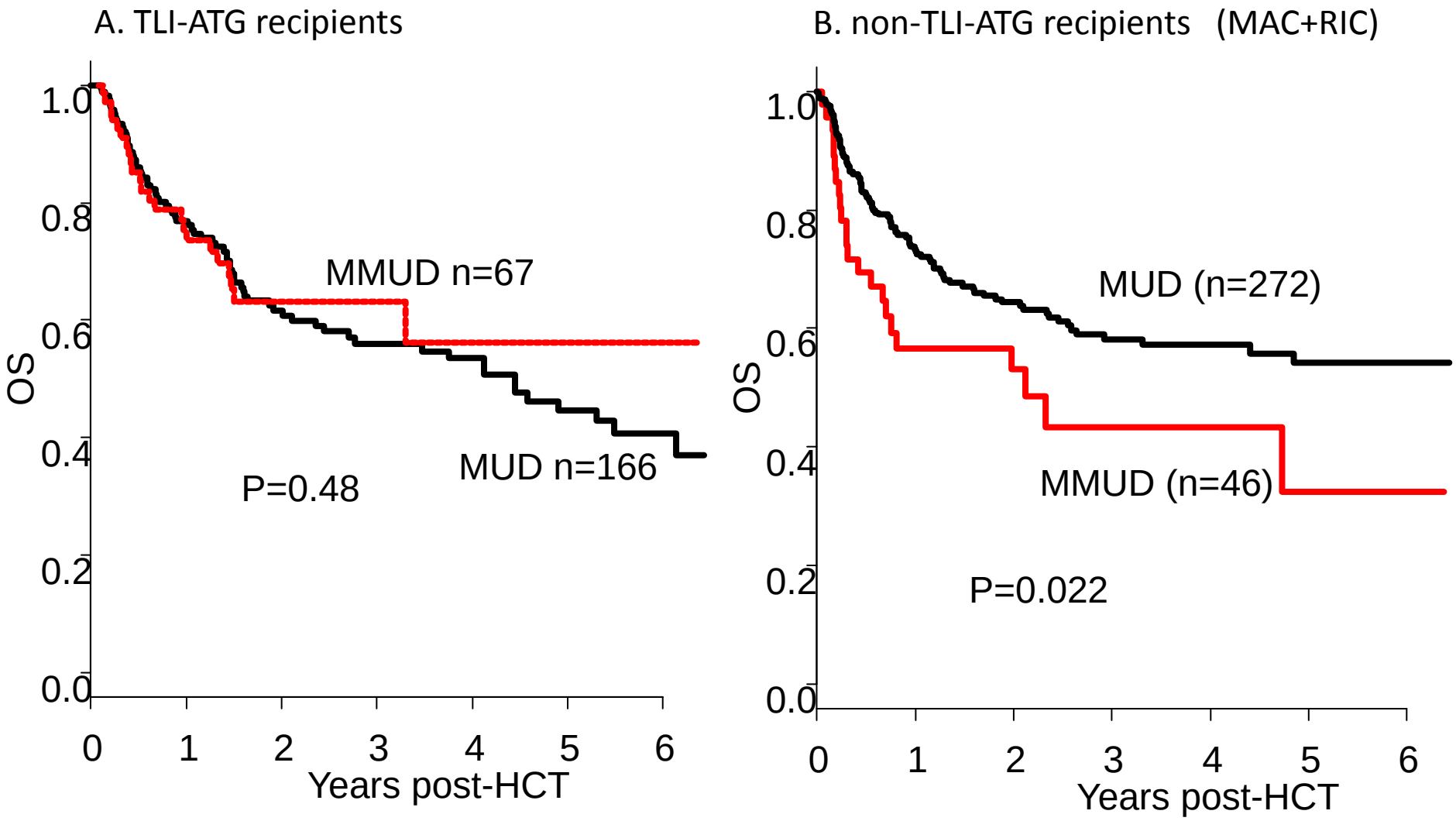

A. TLI-ATG recipients
B. non-TLI-ATG recipients (MAC+RIC)
OS
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Years post-HCT
MMUD n=67
MUD n=166
P=0.48
MUD (n=272)
MMUD (n=46)
P=0.022

Figure S5.

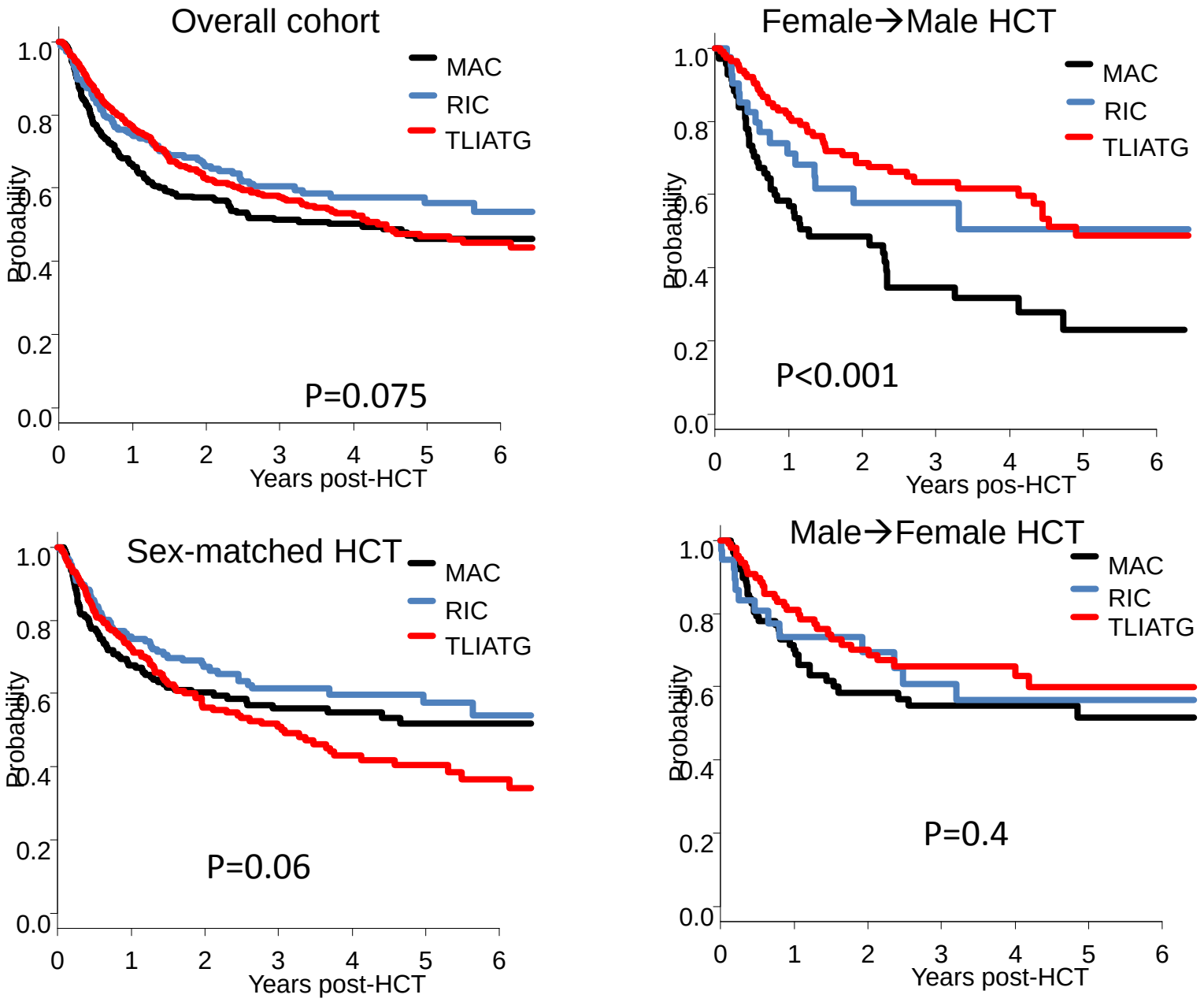
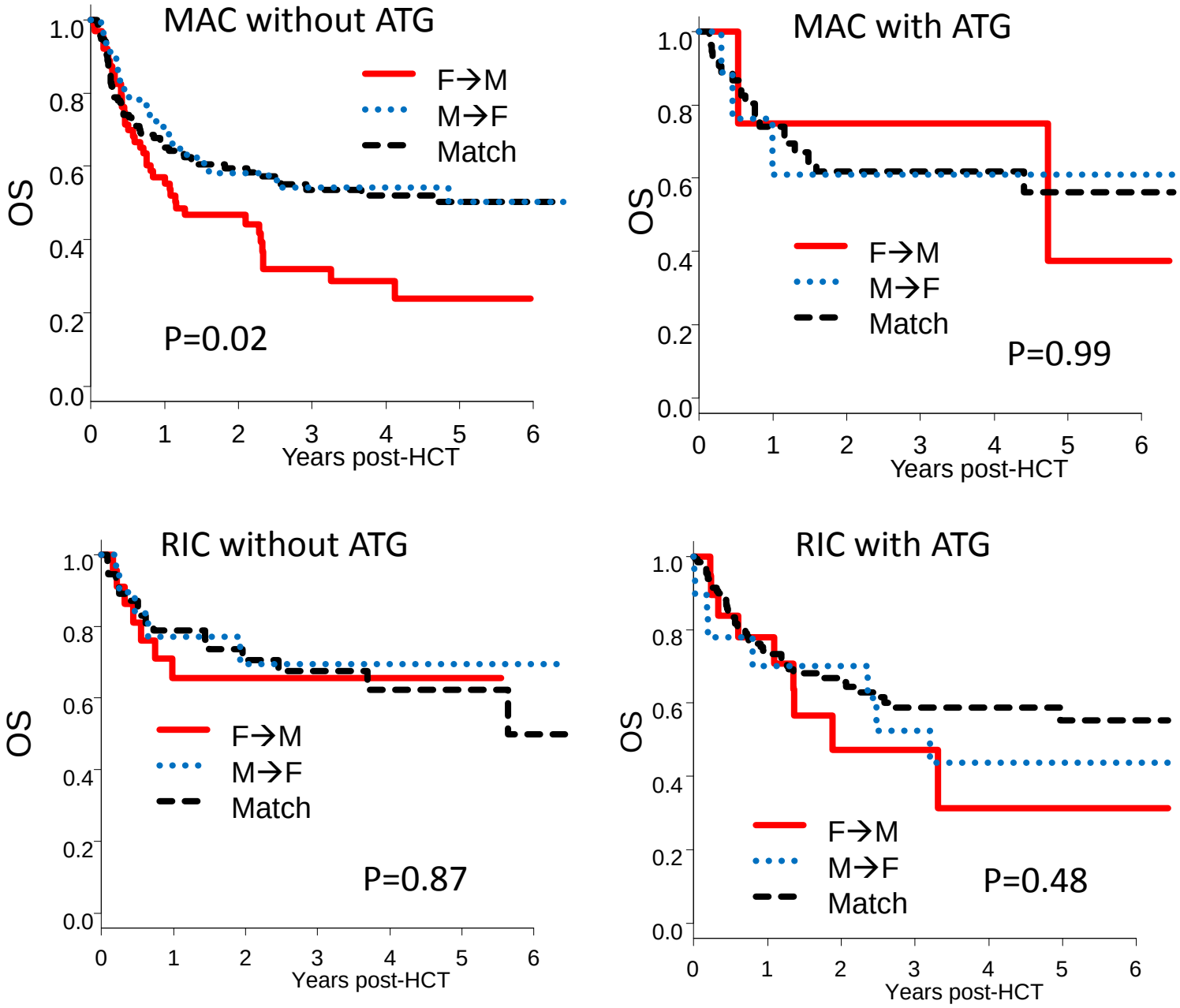

Overall cohort
MAC
RIC
TLIATG
Probability
1.0
0.8
0.6
0.4
0.2
0.0
P=0.075
0
1
2
3
4
5
6
Years post-HCT
Female→Male HCT
MAC
RIC
TLIATG
Probability
P<0.001
Years pos-HCT
Sex-matched HCT
MAC
RIC
TLIATG
Probability
P=0.06
Years post-HCT
Male→Female HCT
MAC
RIC
TLIATG
Probability
P=0.4
Years post-HCT

Figure S6.


MAC without ATG
F→M
M→F
Match
P=0.02
OS
Years post-HCT
MAC with ATG
F→M
M→F
Match
P=0.99
OS
Years post-HCT
RIC without ATG
F→M
M→F
Match
P=0.87
OS
Years post-HCT
RIC with ATG
F→M
M→F
Match
P=0.48
OS
Years post-HCT