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

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ABSTRACT

Sex-mismatched hematopoietic cell transplantation is linked to increased graft-*versus*-host disease and mortality in myeloablative conditioning. Here we evaluated outcomes of 1,041 adult transplant recipients at two centers between 2006 and 2013 and investigated how the effect of sex-mismatching differed in myeloablative, reduced-intensity, and non-myeloablative total lymphoid irradiation with anti-thymocyte globulin conditioning. Among patients who underwent myeloablative conditioning, male recipients with female donors had increased chronic graft-*versus*-host disease (hazard ratio 1.83, $P<0.01$), increased non-relapse mortality (hazard ratio 1.84, $P=0.022$) and inferior overall survival (hazard ratio 1.59, $P=0.018$). In contrast, among patients who received reduced-intensity conditioning, male recipients with female donors had increased acute graft-*versus*-host disease (hazard ratio 1.96, $P<0.01$) but no difference in non-relapse mortality or overall survival. Among the patients who underwent total lymphoid irradiation with anti-thymocyte globulin, male recipients with female donors showed no increase in graft-*versus*-host disease or non-relapse mortality. Notably, only in the cohort receiving total lymphoid irradiation with anti-thymocyte globulin were male recipients with female donors significantly associated with reduced relapse (hazard ratio 0.64, $P<0.01$), and allo-antibody responses against H-Y antigens were predictive of reduced relapse. In the cohort given total lymphoid irradiation with anti-thymocyte globulin, the graft-*versus*-leukemia effect resulted in superior overall survival in recipients of sex-mismatched grafts (HR 0.69, $P=0.037$). In addition, only in the cohort treated with total lymphoid irradiation with anti-thymocyte globulin were female recipients with male donors associated with reduced relapse (hazard ratio 0.59, $P<0.01$) and superior survival (hazard ratio 0.61, $P=0.014$) compared with sex-matched pairs. We conclude that the risks and benefits of sex-mismatched transplants appear to differ according to conditioning strategy and this could affect donor selection.

Introduction

Hematopoietic cell transplantation (HCT) has widely been recognized as a curative procedure for malignant hematologic diseases. However, patients frequently have adverse complications after HCT, including infections, graft-*versus*-host disease (GVHD), and disease relapse. Several factors have so far been identified as risk factors for GVHD or inferior survival: age, disease, disease status, HLA-mismatch, unrelated donors, and cytomegalovirus (CMV) serostatus.[1-6] Among these risk factors, sex-mismatched HCT, especially HCT of male recipients with female donors (Female→Male), is well known to be associated with a higher incidence of GVHD and inferior survival.[1-4,7-10] This adverse effects of Female→Male HCT are thought to result from allogeneic immune responses against minor histocompatibility antigens encoded on the Y-chromosome of a male recipient (H-Y antigens).[7,11-14]

To avoid these post-HCT complications or to reduce disease relapse, various conditioning types have developed.[15] Non-myeloablative conditioning regimens such as total lymphoid irradiation and anti-thymocyte globulin (TLI-ATG) conditioning have shown promise as a method to reduce GVHD incidence and non-relapse mortality.[16-19] TLI-ATG has generally been adapted for older patients or those with comorbidity for the purpose of reducing severe acute GVHD without losing the benefit of allogeneic anti-tumor effects.[16,18] In our experience with the TLI-ATG protocol at Stanford University Hospital, grade II-IV acute GVHD occurred in 10% or less of cases and chronic GVHD in about 30%.[16] These low incidences of GVHD were thought to contribute to the very low non-relapse mortality (less than 10% at 1 year after HCT).[16,17]

Non-myeloablative regimens such as TLI-ATG depend on immunological mechanisms to mediate engraftment and graft-*versus*-leukemia effects. Given the low incidence of non-relapse mortality in TLI-ATG HCT and the generally immune tolerant state that can occur after transplantation, we hypothesized that the increased allo-reactive immunity in sex-mismatched HCT could result in an enhanced graft-*versus*-leukemia/lymphoma (GVL) effect without increased GVHD or non-relapse mortality in the TLI-ATG cohort.

Factors such as recipient age, co-morbidities, disease risk

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factors and disease status are among the major drivers determining the conditioning intensity for HLA-matched or single-allele mismatched transplantation. In general, after selection of conditioning, if multiple potential donors are available, other factors such as blood type and sex of the donor *versus* recipient are evaluated. To determine a potential effect of sex-mismatching within each conditioning type, we evaluated the outcomes of 1,041 adult recipients at two centers between 2006 and 2013. We found that the impact of sex-mismatched transplants differs according to conditioning strategy. Myeloablative conditioned, sex-mismatched Female→Male patients showed increased non-relapse mortality and reduced overall survival compared to sex-matched pairs. In contrast, among patients conditioned with TLI-ATG, sex-mismatched recipients had a lower relapse rate and better overall survival than sex-matched patients. Our results suggest that the selection of potential donors based on their sex depends upon conditioning intensity and that sex-mismatching might be preferred in non-myeloablative TLI-ATG protocols.

Methods

Patients

We reviewed clinical data of 1,041 adults who received peripheral blood stem cell transplants between 2006 and 2013 at Stanford University Hospital (n=749) and Karolinska University Hospital (n=292). Recipients were treated for a number of primary diseases including acute myeloid leukemia, myelodysplastic syndrome, chronic lymphocytic leukemia, non-Hodgkin lymphoma, and others. Our study excluded indications for which TLI-ATG is not used such as acute lymphoblastic leukemia. Haploidentical/HLA-mismatched related-HCT patients and recipients who received GVHD prophylaxis other than cyclosporine and tacrolimus were also excluded.

Plasma samples were collected from recipients who survived without disease relapse for at least 3 months after Female→Male HCT at Stanford University Hospital and were cryopreserved until use. Written informed consent was given for the cryopreservation and analyses of blood samples, in accordance with the Declaration of Helsinki. This study was approved by the institutional review board of Stanford University.

Definitions of categories

The details of category definitions are described in the *Online Supplementary Methods*. Briefly, conditioning regimens were classified into three groups: TLI-ATG, reduced intensity conditioning (RIC), and myeloablative conditioning (MAC). The TLI-ATG conditioning protocol (n=430) has already been reported.[16,18,19] RIC (n=266) mainly included fludarabine-based conditioning regimens. MAC (n=345) included total body irradiation (TBI)-based or busulfan-based conditioning regimens. Donor were also classified into three types: HLA-matched related donor, HLA-matched unrelated donor, and HLA-mismatched unrelated donor. Cord blood transplants were not included in this study. The diagnosis and severity of acute GVHD were reported based on traditional grading scores,[20] while those of chronic GVHD were determined based on the National Institutes of Health 2005 consensus criteria.[21]

Detection of H-Y antibodies in Female→Male hematopoietic cell transplantation

The details are described in the *Online Supplementary Methods*. Briefly, using plasma samples collected 3 months after HCT, antibodies against five H-Y antigens were measured in our proteomic microarray:[22] 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) , and RPS4Y (ribosomal protein S4, Y-linked).

Statistical analysis

The statistical methods are described in detail in the *Online Supplementary Methods*. Briefly, the cumulative probabilities of each event were estimated by the Gray method, considering competing risks. Overall survival from HCT was estimated with a 95% confidence interval (CI) by the Kaplan-Meier method and compared by log-rank test. Since our primary aim was to address the difference of impact of sex-mismatching among different conditioning regimens, we assessed effects of sex-mismatched HCT within the TLI-ATG, RIC, and MAC groups, separately. Multivariate analyses were performed using a Cox proportional hazard model. Since overall survival of sex-matched HCT was not significantly different between Female→Female and Male→Male transplants in the whole cohort, we considered both of these types of transplants as one group of sex-matched HCT. Two-tailed *P*-values <0.05 are considered statistically significant. All analyses and data management were performed using Stata version 12.0 (StataCorp, College Station, TX, USA), and EZR.[23]

Results

Patients' characteristics

In general, conditioning intensity is selected based on patients' characteristics, such as age and co-morbidity, and donor selection follows the choice of conditioning intensity. There are, therefore, many different characteristics, including age, disease, and CMV serostatus that vary among conditioning types (*Online Supplementary Table S1*). For our primary analysis to show clinical significance of sex-mismatched HCT within each conditioning type, we focused on outcomes according to sex-mismatching within the MAC, RIC, and TLI-ATG groups, separately. Within each conditioning group, the patients' characteristics were not very different according to sex-mismatch (Table 1). Within the MAC group, patients with Female→Male HCT were less likely to have received cyclosporine-based GVHD prophylaxis (Table 1). Within the RIC group, patients of sex-matched HCT were less likely to have received grafts from HLA-matched related donors (Table 1). Within the TLI-ATG group, recipients of Male→Female HCT had a higher prevalence of CMV seropositivity (Table 1). The median observed duration of survivors was 957 days.

Overall survival according to sex-mismatch

In agreement with previously published reports, in the MAC group, Female→Male HCT was significantly associated with inferior overall survival compared with the other combinations (HR 1.59; *P*=0.018) (Figures 1A and 2A). In the RIC group, there was no difference in overall survival according to sex-mismatch (Figures 1B, 2A,B).

In the TLI-ATG group, the overall survival of sex-mismatched HCT recipients was better than that of sex-matched HCT recipients (*P*=0.0071) (Figure 1C). Multivariate analyses also confirmed the survival benefit of Female→Male HCT in the TLI-ATG group (HR 0.69; *P*=0.037) (Figure 2A). Focusing on male recipients, a sur-

vival benefit of Female→Male HCT was still observed in the TLI-ATG group (*Online Supplementary Figure S1*). Male→Female HCT was also associated with superior overall survival in the TLI-ATG group (HR 0.61; *P*=0.014) (Figure 2B). However, it did not remain significant when focusing only on female recipients (*Online Supplementary Figure S1*).

In summary, the impact of sex-mismatched HCT on overall survival differed according to conditioning strategy, with worse overall survival in the MAC group and better overall survival in the TLI-ATG group. We next explored what kind of complications could contribute to the sex-mismatched-related difference in overall survival among patients given each type of conditioning.

Incidences of acute and chronic graft-versus-host disease according to sex-mismatch

In the MAC group, there was no difference in incidences of grade II-IV acute GVHD according to sex-mismatch (Figure 3A). In the RIC group, grade II-IV acute GVHD was observed more frequently in sex-mismatched HCT than in sex-matched HCT (Figure 3B). Multivariate analyses also demonstrated that sex-mismatched HCT was associated with an increased risk of grade II-IV acute GVHD in the RIC group (Figure 2A,B), although the adverse impact did not remain statistically significant when only female recipients were considered (*Online Supplementary Figure S1*). In the TLI-ATG group, the incidence of grade II-IV acute GVHD was not different according to sex-mismatch (Figure 3C).

Among patients who survived more than 100 days after HCT without relapse, in the MAC group, Female→Male HCT recipients experienced chronic GVHD more frequently than donor-recipient combinations (*P*=0.041) (Figure 3D). Multivariate analyses also confirmed the adverse effect of Female→Male HCT on chronic GVHD in the MAC group (HR 1.83, *P*=0.0047) (Figure 2A). In contrast, the incidence of chronic GVHD was not different according to sex-mismatch in the RIC and TLI-ATG groups (Figure 3E,F).

In summary, in the TLI-ATG group, sex-mismatched HCT did not have an impact on either grade II-IV acute GVHD or on chronic GVHD, while Female→Male HCT was associated with acute GVHD in the RIC group and chronic GVHD in the MAC group.

Non-relapse mortality according to sex-mismatch

In the MAC group, Female→Male HCT recipients had increased non-relapse mortality compared with donor-recipient combinations (*P*=0.049) (Figure 4A). In the RIC and TLI-ATG groups, there was no difference in non-relapse mortality according to sex-mismatch (Figure 4B,C). Multivariate analyses revealed that the adverse effect of Female→Male HCT was observed only in the MAC group (HR 1.84; *P*=0.022) (Figure 2A), while there was no impact of sex-mismatch within the RIC and TLI-ATG groups (Figure 2A,B).

Table 1. Patients' characteristics according to sex-mismatching within the three different conditioning groups.

	MAC F to M (n=68)	RIC M to F (n=89)	TLI-ATG Matched (n=188)	*P*-value	F to M (n=42)	M to F (n=38)	Matched (n=186)	*P*-value	F to M (n=114)	M to F (n=97)	Matched (n=219)	*P*-value
Age (years)												
18-35	17 (25%)	18 (20%)	38 (20%)	0.46	2 (5%)	3 (8%)	10 (5%)	0.93	7 (6%)	9 (9%)	12 (5%)	0.54
35-50	30 (44%)	49 (55%)	86 (46%)		7 (17%)	5 (13%)	33 (18%)		15 (13%)	11 (11%)	20 (9%)	
50-	21 (31%)	22 (25%)	64 (34%)		33 (79%)	30 (79%)	143 (77%)		92 (81%)	77 (79%)	187 (85%)	
Disease												
AML	36 (53%)	56 (63%)	103 (55%)	0.32	12 (29%)	18 (47%)	64 (34%)	0.18	32 (28%)	25 (26%)	69 (32%)	0.52
Lymphoma or MM	16 (24%)	13 (15%)	29 (15%)		22 (52%)	11 (29%)	70 (38%)		65 (57%)	53 (55%)	106 (48%)	
MDS or MPD	16 (24%)	20 (22%)	56 (30%)		8 (19%)	9 (24%)	52 (28%)		17 (15%)	19 (20%)	44 (20%)	
Disease risk												
standard	30 (44%)	38 (43%)	91 (48%)	0.63	12 (29%)	16 (42%)	65 (35%)	0.45	63 (55%)	58 (60%)	126 (58%)	0.80
high	38 (56%)	51 (57%)	97 (52%)		30 (71%)	22 (58%)	121 (65%)		51 (45%)	39 (40%)	93 (42%)	
CMV status (donor/recipient)												
+/+	28 (41%)	38 (43%)	88 (47%)	0.09	19 (45%)	18 (47%)	92 (49%)	0.52	39 (34%)	40 (41%)	64 (29%)	0.03
-/+	10 (15%)	26 (29%)	52 (28%)		8 (19%)	11 (29%)	48 (26%)		24 (21%)	23 (24%)	43 (20%)	
+/-	10 (15%)	6 (7%)	12 (6%)		6 (14%)	4 (11%)	11 (6%)		26 (23%)	9 (9%)	36 (16%)	
-/-	19 (28%)	19 (21%)	36 (19%)		9 (21%)	5 (13%)	34 (18%)		25 (22%)	25 (26%)	76 (35%)	
ABO mismatch												
Match	42 (62%)	45 (51%)	88 (47%)	0.60	24 (57%)	21 (55%)	84 (45%)	0.47	49 (43%)	58 (60%)	117 (53%)	0.11
Major	12 (18%)	19 (21%)	43 (23%)		6 (14%)	8 (21%)	49 (26%)		25 (22%)	14 (14%)	50 (23%)	
Minor	11 (16%)	21 (24%)	47 (25%)		6 (14%)	7 (18%)	34 (18%)		31 (27%)	20 (21%)	36 (16%)	
Major minor	3 (4%)	4 (4%)	9 (5%)		6 (14%)	2 (5%)	19 (10%)		9 (8%)	5 (5%)	16 (7%)	
Donor												
MRD	41 (60%)	49 (55%)	104 (55%)		23 (55%)	19 (50%)	57 (31%)		57 (50%)	46 (47%)	94 (43%)	
MUD	19 (28%)	33 (37%)	70 (37%)		17 (40%)	17 (45%)	116 (62%)		36 (32%)	40 (41%)	90 (41%)	
MMUD	8 (12%)	7 (8%)	14 (7%)	0.61	2 (5%)	2 (5%)	13 (7%)	0.02	21 (18%)	11 (11%)	35 (16%)	0.33
GVHD prophylaxis												
CsA_based	8 (12%)	22 (25%)	69 (37%)	<0.01	30 (71%)	30 (79%)	140 (75%)	0.74	114 (100%)	97 (100%)	219 (100%)	-
Tac-based	60 (88%)	67 (75%)	119 (63%)		12 (29%)	8 (21%)	46 (25%)		-	-	-	

MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; TLI-ATG: total lymphocyte irradiation with anti-thymocyte globulin; F to M: male recipients with female donors; M to F: female recipients with male donors; AML: acute myelogenous leukemia; MM: multiple myeloma; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; CMV: cytomegalovirus; MRD: HLA-matched related donor; MUD: HLA-matched unrelated donor; MMUD: HLA-mismatched unrelated donor; GvHD: graft-versus-host disease CsA: cyclosporine; Tac: tacrolimus.

In summary, Female→Male HCT was associated with an increased risk of non-relapse mortality in the MAC group, but not in the TLI-ATG or RIC group.

Disease relapse according to sex-mismatch

While no impact of sex-mismatch on relapse was observed in the MAC or RIC group (Figures 2A,B and 4D,E), in the TLI-ATG group, the incidence of relapse was higher following sex-matched HCT than after sex-mismatched HCT (*P*=0.0012) (Figure 4F). Multivariate analyses confirmed that sex-mismatched HCT was significantly associated with a decreased risk of relapse only in the TLI-ATG group (HR 0.64 for Female→Male HCT, *P*=0.0089; HR 0.59 for Male→Female HCT, *P*=0.0075) (Figure 2A,B). Focusing on disease types in the TLI-ATG group, the GVL benefit by sex-mismatching appeared prominent in patients with acute myeloid leukemia (*P*=0.012) and lymphomas (excluding chronic lymphocytic leukemia) (*P*=0.029), while it was not observed in patients with myelodysplastic syndrome or chronic lymphocytic leukemia (*Online Supplementary Figure S2*).

In summary, sex-mismatched HCT was associated with a reduced risk of relapse in the TLI-ATG group, while no association was observed in the MAC or RIC group.

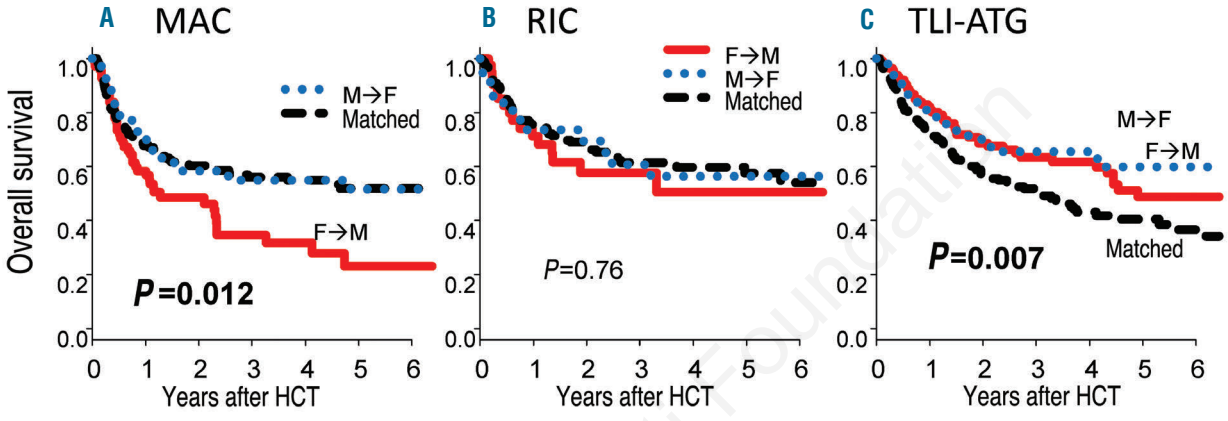

Figure 1. Overall survival according to sex-mismatch in (A) the myeloablative conditioning (MAC), (B) reduced-intensity conditioning (RIC), and (C) total lymphoid irradiation with anti-thymocyte globulin (TLI-ATG) groups.

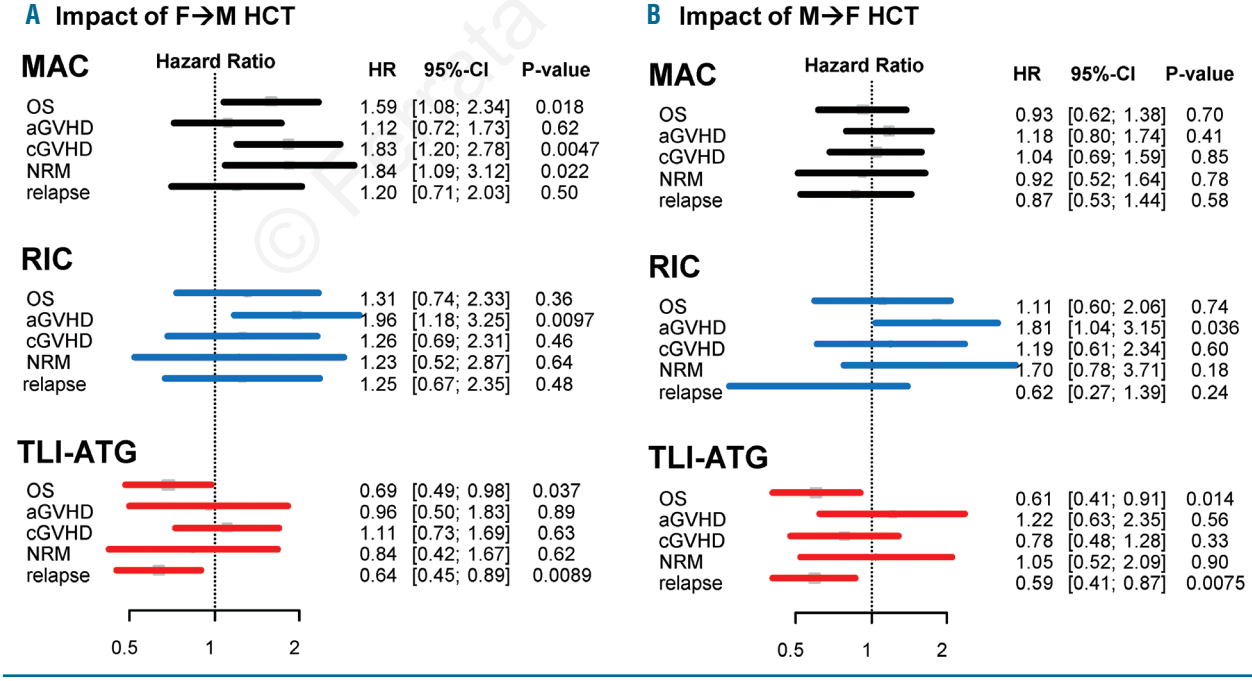

Figure 2. Impact of sex-mismatched transplants on clinical outcomes in comparison with sex-matched transplants in multivariate analyses. Each hazard ratio is shown after adjusting for patient's age, disease, disease risk, patient's cytomegalovirus sero-positivity, and donor type. F→M HCT, transplant of male recipients with female donors; M→F HCT: transplant of female recipients with male donors; TLI-ATG: total lymphoid irradiation with anti-thymocyte globulin; RIC: reduced-intensity conditioning; MAC: myeloablative conditioning; HR: hazard ratio; CI: confidence interval. OS: overall survival; aGVHD: acute graft-*versus*-host disease; cGVHD: chronic graft-*versus*-host disease; NRM: non-relapse mortality.

Detection of H-Y antibodies 3 months after hematopoietic cell transplantation and disease relapse of Female→Male transplant recipients within the group conditioned with total lymphoid irradiation and antithymocyte globulin

In the TLI-ATG group, the benefit of sex-mismatched HCT on overall survival seems due to the reduced relapse rate (Figure 2). We hypothesized that allo-responses through sex-mismatched HCT would contribute to the GVL benefit in the TLI-ATG group. We recently reported H-Y antibody-predicted development of chronic GVHD as an indicator of allo-immunity.[14] We, therefore, focused on the H-Y antibody response in Female→Male HCT and explored whether the detection of H-Y antibodies 3 months after HCT could predict reduced relapse in Female→Male HCT with TLI-ATG.

Excluding patients with myelodysplastic syndrome and chronic lymphocytic leukemia because of the absence of a GVL benefit by sex-mismatch in the TLI-ATG group (*Online Supplementary Figure S2*), H-Y antibodies were tested in a total of 49 TLI-ATG patients of whom half were seropositive (Figure 5A). The incidence of relapse was higher in the recipients who had no H-Y antibody response at 3 months after HCT than in those who did (P=0.037) (Figure 5B). Multivariate analysis corroborated that the detection of H-Y antibodies 3 months after HCT was significantly associated with reduced relapse incidence in Female→Male HCT recipients conditioned with TLI-ATG [HR 0.29 (95% CI: 0.09-0.94), P=0.039] as well as with an increased risk of chronic GVHD [multivariate HR 4.06 (95%CI: 1.03-16.0), P=0.045] (Figure 5C).

H-Y antibodies were also tested for in 28 patients in the MAC group. The distribution of H-Y antibodies was not different between the TLI-ATG and MAC groups (Figure 5A). In the MAC group, relapse incidence was not significantly different according to H-Y seropositivity 3 months after HCT in this cohort (P=0.47) (*Online Supplementary Figure S3A*), while patients who had H-Y antibodies 3 months after HCT experienced more chronic GVHD (83% *versus* 50% at 1 year after HCT, P=0.048 (*Online Supplementary Figure S3B*). In the multivariate analysis of the MAC group, no factors remained statistically significant as risk factors for relapse. However, the number in the MAC group might be too small to enable conclusions to be drawn regarding an absence of association between H-Y antibody development and relapse in this group.

Other assessments

Impact of HLA-mismatch on survival of recipients of unrelated transplants conditioned with total lymphoid irradiation and antithymocyte globulin

Given the results of sex-mismatched transplants, we next assessed whether TLI-ATG patients could benefit from HLA-mismatched HCT or not, focusing on unrelated transplants. In the TLI-ATG group, recipients of HLA-mismatched HCT had comparable overall survival to that of HLA-matched HCT recipients (*Online Supplementary Figure S4A*), while they had inferior overall survival in the other conditioning group, as expected (*Online Supplementary Figure S4B*). The fact that an adverse effect of HLA-mismatch was not found in the TLI-ATG group suggests that the ATG-based conditioning may be safer for some patients with HLA-mismatched donors, although we did not see a benefit in terms of decreased relapse risks as we

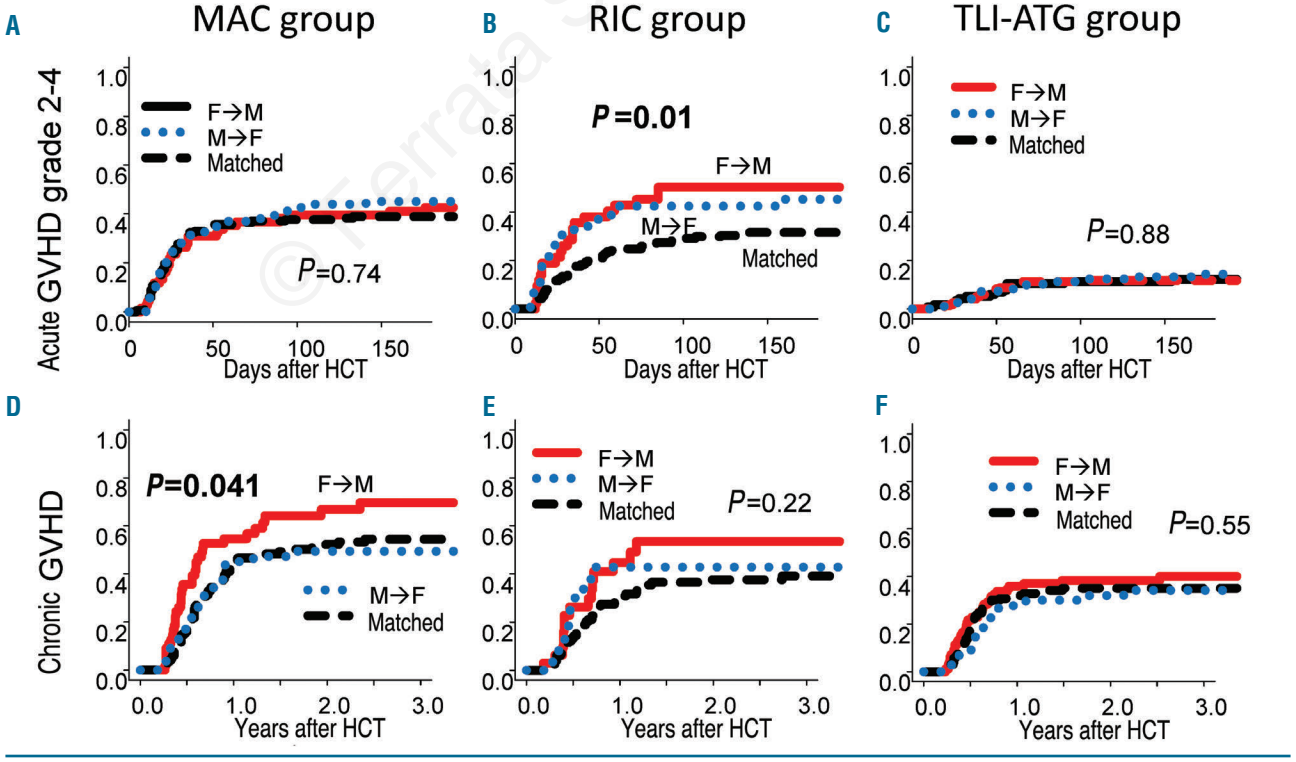

Figure 3. Incidences of acute GVHD grade 2-4 (upper figures) and chronic GVHD (lower figures) according to sex-mismatch in (A&D) the group conditioned with total lymphoid irradiation with anti-thymocyte globulin (TLI-ATG), (B&E) the reduced-intensity conditioning (RIC) group, or (C&F) the myeloablative conditioning (MAC) group.

saw in sex-mismatched HCT. Further investigation is required before drawing any conclusions.

Impact of conditioning strategies within the group of sex-mismatched hematopoietic cell transplant recipients

Many clinical parameters, such as age, disease, and co-morbidity drive clinicians to select conditioning intensity before considering the choice of donor. We, therefore, decided to base the bulk of our evaluation on sex-mismatched HCT within the three conditioning intensity regimens. Nonetheless, we next evaluated the effects of conditioning intensity. In the overall cohort, recipients in the TLI-ATG group had comparable overall survival to those in the MAC group (*Online Supplementary Figure S5*). Looking at the subgroups stratified by sex-mismatch, the TLI-ATG group had superior overall survival in Female→Male HCT (*Online Supplementary Figure S5*). Multivariate analysis also demonstrated that TLI-ATG was associated with favorable outcome in the Female→Male HCT group, but not in sex-matched or Male→Female HCT (*Online Supplementary Table S2*). However, it should be noted that the backgrounds were very different among conditioning strategies (*Online Supplementary Table S1*), which could have affected the results.

Discussion

In general, conditioning intensity is selected based on age, co-morbidity, and disease status. Since donor selection usually follows the choice of conditioning intensity, the effect of having a sex-mismatched donor should be considered within each conditioning strategy. We found that sex-mismatched HCT, especially Female→Male HCT, was an important factor affecting survival, GVHD, and relapse, and that the impact differed according to conditioning strategy and warrants consideration in donor selection algorithms. Specifically, we found that patients undergoing TLI-ATG conditioning benefit from sex-mismatched GVL allo-immunity without the detrimental non-relapse mortality associated with GVHD.

It has been widely reported that Female→Male HCT is associated with an increased incidence of chronic GVHD and decreased overall survival.[1-4] However, as summarized in Figure 6, our study shows that the impact of sex-mismatched HCT differs depending on the conditioning regimen used. In agreement with prior reports, our MAC analysis showed that Female→Male HCT was associated with inferior survival due to increased non-relapse mortality and chronic GVHD. However, within our group of patients given RIC, Female→Male HCT did not affect overall survival despite its association with an increased incidence of acute GVHD. Unexpectedly, within our TLI-ATG group, Female→Male HCT was associated with a better overall survival (HR 0.69 relative to sex-matched HCT; *P*=0.037), because of reduced relapses, but had no impact on either GVHD or non-relapse mortality. The incidence of acute GVHD is relatively low in TLI-ATG recipients, compared to recipients of most other conditioning protocols, because of the minimization of organ damage to GVHD-prone epithelial tissues and/or a tolerogenic immune state, resulting in

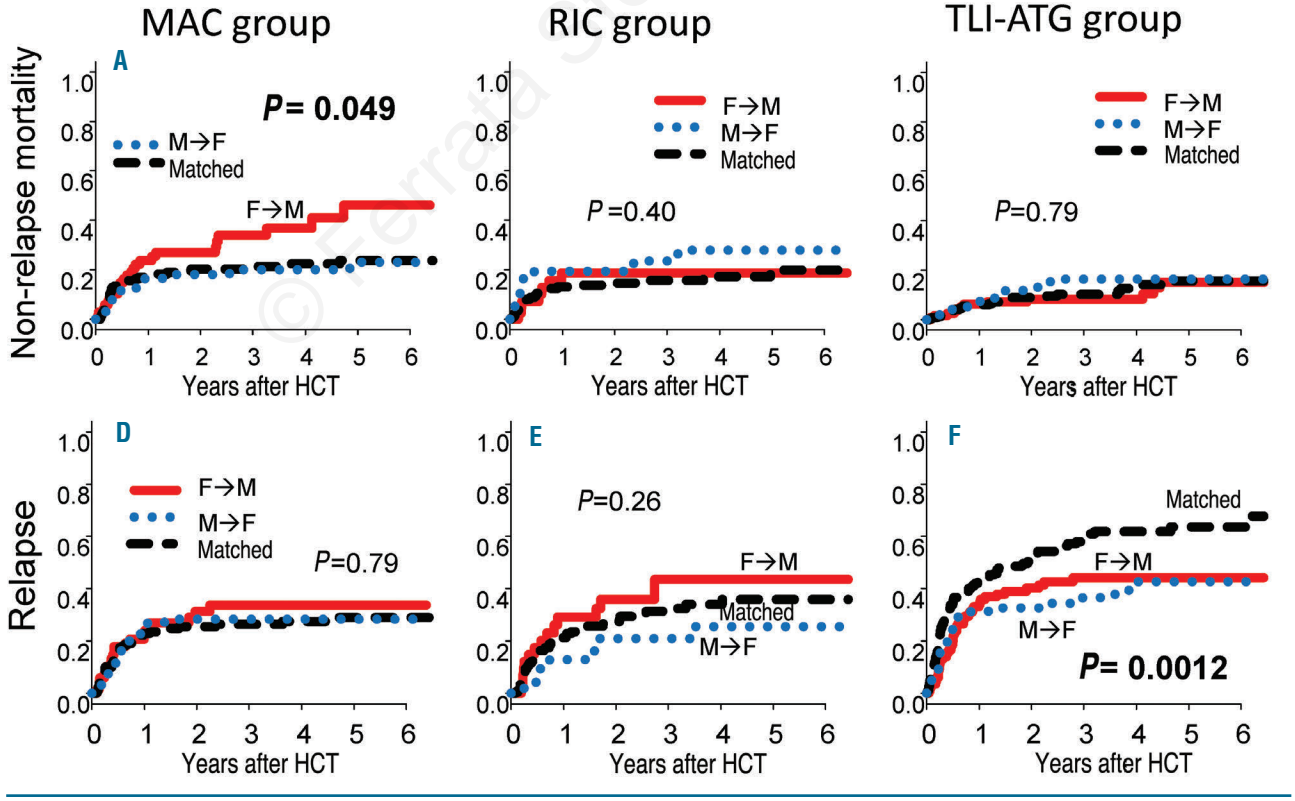

Figure 4. Non-relapse mortality (upper figures) and relapse incidences (lower figures) according to sex-mismatch in the group (A&D) the group conditioned with total lymphoid irradiation with anti-thymocyte globulin (TLI-ATG), (B&E) the reduced-intensity conditioning (RIC) group, and (C&F) the myeloablative conditioning (MAC) group.

low non-relapse mortality.[16-18] In addition to the low non-relapse morality, TLI-ATG patients seem to benefit from the GVL effect by sex-mismatch, leading to a survival benefit, which appears unique to TLI-ATG.

Since TLI-ATG is devoid of chemotherapy cytotoxicity, conditioning provides little direct anti-tumor benefit. Rather, the successful outcome of TLI-ATG transplants depends mostly on an allo-immune GVL benefit. We hypothesized that the increased allo-reactive immunity in sex-mismatched HCT could result in an enhanced GVL effect without increased GVHD or non-relapse mortality in the TLI-ATG cohort. The apparent GVL benefit from sex-mismatched donors in the TLI-ATG group supports our hypothesis and will be considered during donor selection. Pre-clinical studies in animal models have suggested that TLI-ATG can increase the proportion of host natural killer T cells, leading to expansion of donor $CD4^+CD25^+Foxp3^+$ regulatory T cells through interleukin-4 pathways.[24,25] These regulatory T cells are thought to have a potential not only to suppress fatal acute GVHD but also to preserve the anti-tumor effect of cytotoxic T cells.[26,27] Future studies of allo-reactive cell populations could help to clarify the immune dynamics that account for the GVL benefit by sex-mismatched HCT in TLI-ATG patients.

H-Y humoral immunity is an allogeneic response of female donors against the proteins encoded on the Y chromosome of male recipients and is considered an important model of allo-immunity.[7,14] To relate sex-mismatched allo-immunity more directly with decreased relapse, we measured H-Y antibody development in Female→Male HCT 3 months after the transplant. About half of TLI-ATG patients had developed at least one H-Y antibody, and an association was observed between H-Y antibody development and chronic GVHD, as we previously reported.[14] Importantly, detection of H-Y alloantibodies 3 months after HCT was significantly associated with reduced relapse incidence (Figure 5), demonstrating an association between allo-response in Female→Male HCT and GVL benefit in the TLI-ATG group. In the future, a more detailed study of humoral immunity and T-cell response might illuminate mechanisms of enhanced GVL without increased GVHD or non-relapse mortality.

While Female→Male HCT is an established risk factor for chronic GVHD in the MAC group, the impact of sex-mismatching on acute GVHD has been controversial.[1,2,28] In our cohort, an association of sex-mismatching with an increased risk of acute GVHD was observed only in the RIC group. Many factors can potentially contribute to the onset of acute GVHD and it is possible that differences in conditioning intensity, prophylactic immunosuppression and/or immune reconstitution may contribute to the timing and onset of acute GVHD.

Our results show that the improved GVL benefit in the TLI-ATG group associated not only with Female→Male but also Male→Female HCT. The concept of female-specific gene expression has been suggested before and might play a role here. For example, there may be several genes expressed only in female cells, such as *XIST*, a gene that

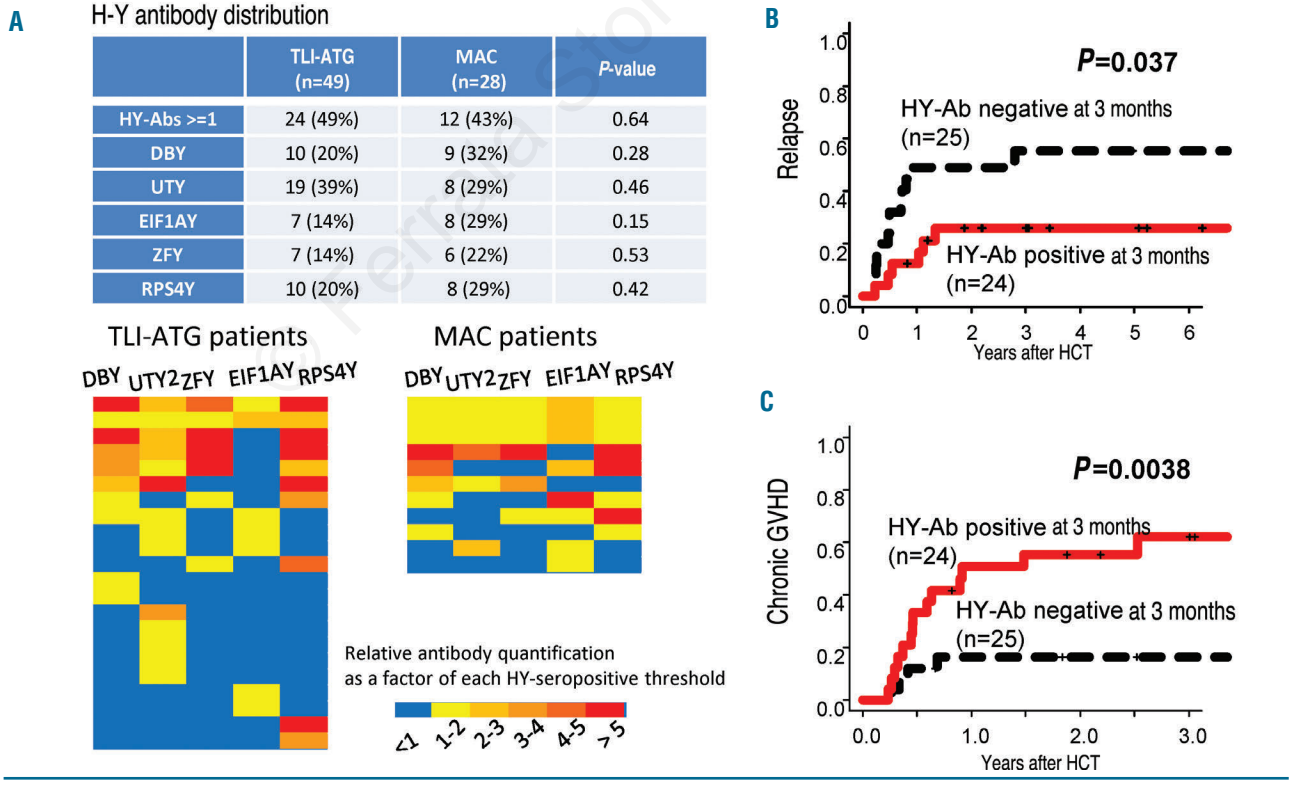

Figure 5. Impact of H-Y antibody detection 3 months after transplantation. (A) H-Y antibody (Ab) distributions of the two groups conditioned with total lymphoid irradiation with anti-thymocyte globulin (TLI-ATG) and myeloablative conditioning (MAC) regimens. In the heat-map, only patients testing H-Y seropositive are shown. The heat-map indicates relative antibody quantification as a factor of each H-Y-seropositive threshold. UTY2 are shown as representative of three fragments of UTY1-3. (B) Disease relapse according to the detection of H-Y antibodies 3 months after Female→Male transplant in the group, excluding patients with myelodysplastic syndrome and chronic lymphocyte leukemia. (C) Chronic graft-*versus*-host disease (GVHD) according to the detection of H-Y antibodies 3 months after Female→Male transplant in the TLI-ATG group.

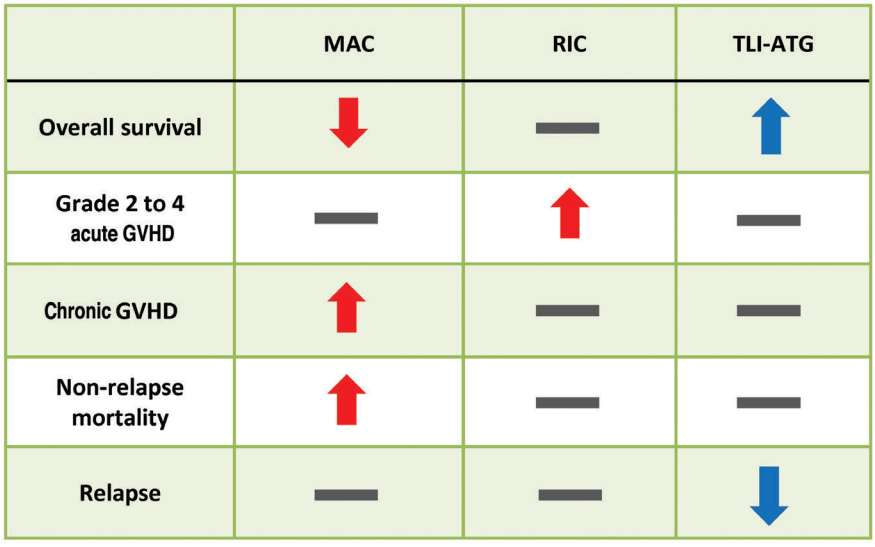

Figure 6. Summary of clinical outcomes of Female→Male transplantation within each conditioning group. Blue arrows indicate a favorable effect and red arrows indicate an adverse effect, compared with sex-matched transplantation. Crossbars denote similar outcomes compared with sex-mismatched transplantation.

inactivates one of the X chromosomes, which is not observed in male cells.[29-31] In addition, inactivated X chromosome has recently been reported to accumulate hypermutation in various tumor cells.[32] These phenomena, observed exclusively in females, may become candidate anti-tumor immune targets under a unique T-cell environment induced by TLI-ATG, although the real reason for the GVL benefit from Male→Female HCT remains unclear. One potential confounder to consider is that in the TLI-ATG group, the rate of CMV seropositivity tended to be higher in Male→Female HCT patients (Table 1). CMV-seropositive patients were associated with early CMV reactivation in TLI-ATG.[33] CMV reactivation has been reported to be associated with a reduced risk of relapse, but only in patients with acute myeloid leukemia and not in those with lymphoma.[34,35] Unless the mechanism of CMV in enhancing GVL manifests differently in TLI-ATG, the enhanced GVL is likely to be related to the sex-mismatch.

Our study may have some limitations because of its retrospective nature. The study cohort had heterogeneous disease backgrounds and relapse incidence might depend on diseases and their status. We, therefore, explored the impact of sex-mismatch in each disease category as a subgroup analysis and found that the GVL benefit by sex-mismatch of TLI-ATG was limited in specific diseases such as acute myeloid leukemia and lymphoma other than chronic lymphocytic leukemia. One possible explanation is that expression of H-Y antigens might vary according to type of disease or individual leukemic cells. Loss of the Y chromosome is frequently observed in myelodysplastic syndrome (10-15%).[36,37] The defect of H-Y antigens in tumor cells with loss of Y chromosome may result in a reduced GVL effect by sex-mismatch, although the real reason has yet to be elucidated. Furthermore, MAC and RIC groups included various conditioning regimens and ATG usage in these conditioning regimens may also affect the impact of sex-mismatch. MAC Female→Male recipients had equivalent survival as sex-matched recipients when ATG or alemtuzumab was included (*Online Supplementary Figure S6*). In contrast, MAC Female→Male recipients who did not receive ATG or alemtuzumab had significantly worse survival than that of sex-matched recipients ($P<0.01$) (*Online Supplementary Figure S6*). In cases in which a sex-mismatched transplant is the only option in patients who have received MAC, our results suggest that the risks of sex-mismatching might be reduced by incorporating ATG into the conditioning regimen, although larger retrospective cohort studies or prospective studies are needed to be certain.

In summary, the benefits and risks of sex-mismatched transplants differ according to conditioning strategy. Recipients of TLI-ATG conditioning preferentially benefit from sex-mismatched HCT with significantly reduced relapse rates and improved overall survival. The presence of H-Y antibodies 3 months after HCT, as representative alloantibodies demonstrated the association with reduced relapse in the TLI-ATG group. This could affect donor selection, such that sex-mismatching might be preferred in TLI-ATG patients and avoided in myeloablative transplant recipients. Our results suggest that there is the possibility of developing and employing new strategies to enhance GVL effects in TLI-ATG and non-myeloablative conditioning without increasing toxicity.

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Authorship and Disclosures

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