Deletion of Y-chromosome before allogeneic hematopoietic stem cell transplantation in male recipients with female donors

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Abstract:
The graft-versus-leukemia (GVL) effect is one of the curative mechanisms of allogeneic hematopoietic stem cell transplantation (allo-HCT). H-Y antigens, which are encoded by Y-chromosome, are important targets of the GVL. Thus, Deletion of Y-chromosome (the Del(Y) group) might deteriorate the GVL in a transplant between a female donor and male recipient, although the clinical significance of the Del(Y) group remains to be elucidated. In this study, we evaluated adult male patients who received allo-HCT between 2010 and 2019 in Japan. There were 155 cases in the Del(Y) group and 4149 cases without Del(Y) who received female-to-male allo-HCT. The Del(Y) group was significantly associated with inferior overall survival (HR 1.24 [95% CI: 1.00 - 1.53], P = 0.049) and an increased risk of relapse (HR 1.40 [95% CI: 1.08 - 1.80], P = 0.0098) in multivariate analyses. There was no significant difference in non-relapse mortality between recipients with and without Del(Y) (HR 1.40 [95% CI: 0.769 - 2.61], P = 0.67). On the other hand, the Del(Y) group was not significantly associated with any clinical outcomes in the cohort of male-to-male allo-HCT. A higher incidence of relapse might be caused by attenuation of the GVL due to a lack of H-Y antigens. Since a GVL due to a sex-mismatch itself may not be expected in male recipients with Del(Y) who receive allo-HCT from a female donor, additional post-allo-HCT strategies might be required to prevent disease relapse.

Conflict of interest: COI declared - see note

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Key points

- Deletion of Y-chromosome before transplantation was significantly associated with disease relapse in female-to-male allo-HCT.

- A higher incidence of relapse in the Del(Y) group might be caused by attenuation of GVL due to a lack of H-Y antigens.

Abstract

The graft-versus-leukemia (GVL) effect is one of the curative mechanisms of allogeneic hematopoietic stem cell transplantation (allo-HCT). H-Y antigens, which are encoded by Y-chromosome, are important targets of the GVL. Thus, Deletion of Y-chromosome (the Del(Y) group) might deteriorate the GVL in a transplant between a female donor and male recipient, although the clinical significance of the Del(Y) group remains to be elucidated. In this study, we evaluated adult male patients who received allo-HCT between 2010 and 2019 in Japan. There were 155 cases in the Del(Y) group and 4149 cases without Del(Y) who received female-to-male allo-HCT. The Del(Y) group was significantly associated with inferior overall survival (HR 1.24 [95% CI: 1.00 – 1.53], P = 0.049) and an increased risk of relapse (HR 1.40 [95% CI: 1.08 – 1.80], P = 0.0098) in multivariate analyses. There was no significant difference in non-relapse mortality between recipients with and without Del(Y) (HR 1.08 [95% CI: 0.769 – 1.51], P = 0.67). On the other hand, the Del(Y) group was not significantly associated with any clinical outcomes in the cohort of male-to-male allo-HCT. A higher incidence of relapse might be caused by attenuation of the GVL due to a lack of H-Y antigens. Since a GVL due to a sex-mismatch itself may not be expected in male recipients with Del(Y) who receive allo-HCT from a female donor, additional
post-allo-HCT strategies might be required to prevent disease relapse.
Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HCT) is a curative treatment approach for hematological malignancies, although it is associated with high morbidity and mortality. One of the curative mechanisms of allo-HCT involves the graft-versus-leukemia (GVL) effect, which is an immune reaction mediated by allo-reactive donor lymphocytes, although a favorable effect of GVL has not been clearly elucidated due to its robust relationship with graft-versus-host disease (GVHD). While the main targets of the GVL effect are human leukocyte antigens, minor histocompatibility antigens are also important.

H-Y antigens, which are proteins encoded by Y-chromosome, are important minor histocompatibility antigens. Y-chromosome is the sex-determining chromosome and contains several genes that are involved in the differentiation of male-specific organs, spermatogenesis, various cytokines and the cell cycle. Since Y-chromosome is specific to males, H-Y antigens are potential targets of GVHD in transplants between female donors and male recipients (female-to-male allo-HCT). A combination of male recipient and female donor has been associated with an increased risk of GVHD and inferior survival, but a lower relapse rate in female-to-male allo-HCT in selected situations suggested that H-Y antigens may play an important role as a target of the GVL effect. In this regard, deletion of Y-chromosome (Del(Y)) in tumor cells might reduce this favorable effect of GVL. Del(Y) is a common mutation in somatic cells that increases with aging. However, the significance of Del(Y) in the GVL effect remains to be elucidated. Moreover, Del(Y) has been associated with age-related diseases such as cancer, cardiovascular disease, Alzheimer’s disease and diabetes. Although these results suggest a possible relationship...
between Del(Y) and several allo-HCT complications, the clinical significance of Y-chromosome in allo-HCT other than GVHD and GVL has been poorly elucidated. In this study, we evaluated the clinical impact of Del(Y) on clinical outcomes in female-to-male allo-HCT patients.

Methods

Data source and patient selection

Clinical data were provided by the Transplant Registry Unified Management Program (TRUMP) of the Japanese Society for Transplantation and Cellular Therapy (JSTCT) and the Japanese Data Center for Hematopoietic Cell Transplantation (JDCHCT) \(^{14,15}\). This study included adult male patients (age ≥ 16 years) who were diagnosed as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN), and who received their first allo-HCT between January 2010 and December 2019 in Japan. Patients who lacked information on karyotype at allo-HCT or diagnosis were excluded. This retrospective study was approved by the data management committee of TRUMP and by the Institutional Review Board of Jichi Medical University Saitama Medical Center.

Definitions

Del(Y) was evaluated by G-banding karyotyping with a bone marrow specimen at allo-HCT or at the diagnosis, since karyotyping data only at the diagnosis were reported in all of the patients except for those with MDS. All patients with deletion of Y-chromosome were classified as the Del(Y) group and the others were classified as the Y-present group. Any additional chromosomal abnormalities
were permitted.

Disease risk index (DRI) and hematopoietic cell transplantation comorbidity index (HCT-CI) were assessed based on a previous report 16,17. A low disease risk of DRI included AML with favorable cytogenetics, whereas a high disease risk of DRI included AML and MDS with adverse cytogenetics. The other diseases were classified as intermediate disease risk. A high stage risk of DRI included induction failure and active relapse at transplantation, and any other disease status was classified as low stage risk. In terms of cytogenetics, for AML, t(8;21), inv(16) and t(15;17) were classified as favorable, complex karyotype (≥4 abnormalities) as adverse and other karyotypes as intermediate. For MDS, abnormal chromosome 7 and complex karyotype (≥4 abnormalities) were classified as adverse and the other karyotypes were considered intermediate 16. Conditioning regimens were classified as either myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) according to the criteria from the Center for International Blood and Marrow Transplant Research 18. In brief, MAC regimens were defined by total body irradiation > 8 Gy (fractionated) or ≥ 5 Gy (single dose), intravenous busulfan ≥ 7.2 mg/kg, oral busulfan ≥ 9 mg/kg, or melphalan > 140 mg/m2, whereas other regimens were defined as RIC. T cell in vivo depletion included anti-thymocyte globulin and alemtuzumab. A related donor with 6/6 antigen matches of HLA-A, -B and -DR was considered to be an HLA-matched related donor (MRD), and any other related donors were considered to be an HLA-mismatched related donor (MMRD). In bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT), an unrelated donor with 8/8 allelic matches of HLA-A, -B, -C and -DR was classified as an HLA-matched unrelated donor (MUD), whereas any other voluntary donors were classified as an...
HLA-mismatched unrelated donor (MMUD). In cord blood transplantation (CBT), a cord blood unit with 6/6 antigen matches of HLA-A, -B and -DR was considered to be MUD, and all other cord blood units were considered to be MMUD. Acute and chronic GVHD were diagnosed and graded based on standard criteria\textsuperscript{19,20}.

**Statistical analysis**

Statistical analyses were mainly performed for female-to-male recipients. Patient characteristics were compared between the Del(Y) and Y-present groups using Fisher’s exact test for categorical variables and the Mann-Whitney U test for continuous variables. The cumulative incidences of non-relapse mortality (NRM), relapse, grade II - IV acute GVHD and chronic GVHD were estimated by Gray’s test and compared between the Del(Y) and Y-present group. Relapse and NRM were treated as competing risks for each other. Death due to any cause was treated as a competing risk for the other cumulative incidences. The cumulative incidence of chronic GVHD was evaluated among patients who survived more than 100 days after allo-HCT. Overall survival (OS) was estimated by the Kaplan-Meier method and compared by the log-rank test. A Cox proportional hazard regression model was used for multivariate analyses of survival outcomes and cumulative incidences of GVHD. Covariates included in the multivariate analyses were age, disease type, DRI, HCT-CI, performance status (PS), donor type, conditioning intensity, GVHD prophylaxis, and T cell in vivo depletion.

In addition, the impact of Del(Y) was compared in a cohort matched for background factors such as age (> 50 years or ≤ 50 years), disease type (AML, ALL, MDS or MPN), DRI (low, intermediate,
high or very high), HCT-CI ($\geq 2$ or $0 – 1$), PS ($2 – 4$ or $0 – 1$), donor type (matched related, matched unrelated, mismatched related or mismatched unrelated), donor source (BMT, PBSCT or CBT), conditioning regimen (MAC or RIC), GVHD prophylaxis type (cyclosporin based, tacrolimus based or others) and T cell in vivo depletion (with or without anti-thymocyte globulin), using caliper widths equal to 0.2 standard deviation.

A two-tailed P value <0.05 was considered to be statistically significant. After the analyses for female-to-male patients, analyses for male-to-male patients were performed in the same manner to validate the effect of Del(Y) on female-to-male transplant patients. All analyses in this study were performed with EZR (https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html, Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R version 3.6.3 (The Foundation for Statistical Computing, Vienna, Austria) \(^21\).

**Results**

**Patient characteristics of female-to-male allo-HCT recipients**

According to the eligibility criteria of this study, 155 cases in the Del(Y) group and 4149 cases in the Y-present group were evaluated in the analyses for female-to-male allo-HCT recipients. The median duration of follow-up for survivors was 41 months ($0 – 127$). The median recipient age for the entire cohort was 53 years ($16 – 85$). The median age was 56 years ($18 – 77$) in the Del(Y) group and 53 years ($16 – 85$) in the Y-present group. Patient characteristics of the Del(Y) and Y-present groups are shown in Table 1. In the Del(Y) group, 18 cases lacked Y-chromosome without any additional chromosomal abnormalities. The Del(Y) group included more patients with AML, fewer with ALL and those with a higher HCT-CI score. DRI of the Del(Y) group was distributed...
equally among all risk groups, whereas the Y-present group included fewer “low” and “very high” patients. There was no significant difference in age between the Del(Y) and Y-present groups.

**Survival outcomes and incidence of GVHD for female-to-male transplant patients**

OS was significantly inferior in the Del(Y) group (5y-OS 35.1% vs 41.6%, \( P < 0.001 \), Figure 1a). Multivariate analysis confirmed a significant relationship between Del(Y) and inferior OS (hazard ratio \( [HR] 1.25 \) [95% confidence interval \( [CI] \): 1.01 – 1.54], \( P = 0.042 \), Figure 2). While NRM was comparable between the Del(Y) and Y-present groups (5y-NRM 24.4% vs 29.4%, \( P = 0.64 \), Figure 1b), the cumulative incidence of relapse (CIR) was significantly higher in the Del(Y) group (5y-CIR 48.4% vs 31.6%, \( P < 0.001 \), Figure 1c). Multivariate analysis of NRM and CIR was consistent with the results of the univariate analysis (HR of NRM 1.09 [95% CI: 0.778 – 1.52], \( P = 0.62 \); HR of CIR 1.40 [95% CI: 1.08 – 1.80], \( P = 0.010 \), Figure 2).

The cumulative incidences of acute and chronic GVHD were similar in the Del(Y) and Y-present groups (5y-grade II - IV acute GVHD 35.5% vs 33.5%, \( P = 0.63 \); 5y-chronic GVHD 33.1% vs 39.4%, \( P = 0.12 \)). The multivariate analysis did not show a significant relationship between Del(Y) and either grade II - IV acute GVHD (HR 1.18 [95% CI: 0.890 – 1.56], \( P = 0.25 \)) or chronic GVHD (HR 0.819 [95% CI: 0.576 – 1.16], \( P = 0.27 \)) (Supplemental Table 1).

Death due to disease progression was more frequent in the Del(Y) group (32.9% vs 19.0%, \( P < 0.001 \)), while other causes of death such as infection and acute GVHD were comparable between the two groups (infection 8.4% vs 10.0%, \( P = 0.59 \); acute GVHD 1.3% vs 2.1%, \( P = 0.77 \), Supplemental Table 2).
Subgroup analyses according to HLA disparity between male recipients and female donors

In the HLA-matched donor cohort (male recipients with HLA-matched related or unrelated female donors, n = 1757), the Del(Y) group showed inferior OS and CIR than the Y-present group (5y-OS 28.6% vs 46.4%, P <0.001; 5y-CIR 51.0% vs 31.8%, P <0.001). Multivariate analyses also showed that Del(Y) was significantly associated with inferior OS and an increased risk of CIR (HR for OS 1.63 [95% CI: 1.19 – 2.24], P = 0.0026; HR for CIR 1.61 [95% CI: 1.10 – 2.35], P = 0.015, Supplemental Figure 1). NRM was comparable between the Del(Y) and Y-present groups (5y-NRM 23.7% vs 24.9%, P = 0.56). Multivariate analysis also showed no significant relationship between Del(Y) and NRM (HR 1.47 [95% CI: 0.87 – 2.49], P = 0.15).

On the other hand, in the HLA-mismatched donor cohort (male recipients with HLA-mismatched related or unrelated female donors, n = 2435), the Del(Y) group tended to show inferior OS than the Y-present group (5y-OS 37.9% vs 38.4%, P = 0.098), and CIR was also inferior in the Del(Y) group (5y-CIR 46.8% vs 31.2%, P = 0.0058). However, the differences did not remain significant in multivariate analyses (HR for OS 1.05 [95% CI: 0.79 – 1.39], P = 0.75; HR for CIR 1.31 [95% CI: 0.927 – 1.84], P = 0.13, Supplemental Figure 1). NRM was comparable between the Del(Y) and Y-present group (5y-NRM 25.6% vs 32.7%, P = 0.41; HR of Del(Y) 0.89 [95% CI: 0.57 – 1.38], P = 0.60). In summary, the impact of Del(Y) on survival and CIR seems apparent in the HLA-matched female-to-male allo-HCT, and anti-HLA alloreactivity may outweigh responses against minor antigens in the HLA-mismatched female-to-male allo-HCT.
Matched-pair analysis of survival outcomes and GVHD in female-to-male allo-HCT recipients according to Del(Y)

Since there was a considerable difference in background between the Del(Y) and Y-present groups, a matched-pair analysis was performed. As a result, 117 cases per group were matched (Supplemental Table 3).

In the matched-pair analysis, OS was comparable between the Del(Y) and Y-present groups (5y-OS 32.5% vs 38.5%, P = 0.14, Figure 3a). There was also no significant difference in NRM between the Del(Y) and Y-present groups (5y-NRM 28.5% vs 34.1%, P = 0.67, Figure 3b), whereas CIR was significantly inferior in the Del(Y) group (5y-CIR 45.8% vs 30.8%, P = 0.037, Figure 3c).

With regard to GVHD, there was no significant difference in the cumulative incidences of grade II-IV acute GVHD and chronic GVHD between the two groups (5y-grade II - IV acute GVHD 41.9% vs 35.9%, P = 0.32; 5y-chronic GVHD 31.9% vs 36.7%, P = 0.42).

Survival outcomes and incidence of GVHD in male-to-male allo-HCT recipients

We additionally analyzed male-to-male allo-HCT recipients to check whether or not the adverse impact of Del(Y) on relapse was observed only in female-to-male allo-HCT.

The Del(Y) group (n = 225) showed significantly inferior OS and CIR compared to the Y-present group (n = 6399) in a univariate analysis (5y-OS 40.3% vs 46.5%, P = 0.0043; 5y-CIR 35.3% vs 29.9%, P = 0.047), whereas NRM was comparable between the Del(Y) and Y-present groups (5y-NRM 26.5% vs 26.9%, P = 0.60). The cumulative incidences of acute and chronic GVHD in the Del(Y) and Y-present groups were similar (5y-grade II - IV acute GVHD 38.0% vs 38.8%, P = 0.67;
5y-chronic GVHD 32.4% vs 37.1%, P = 0.35). However, multivariate analyses did not show any significant relationship between Del(Y) and clinical outcomes (HR of OS 1.15 [0.954 – 1.38], P = 0.15; HR of NRM 1.08 [95% CI: 0.824 – 1.43], P = 0.56; HR of CIR 1.17 [95% CI: 0.925 – 1.49], P = 0.19; HR of grade II - IV acute GVHD 0.963 [95% CI: 0.772 – 1.20], P = 0.74; HR of chronic GVHD 0.882 [95% CI: 0.669 – 1.16], P = 0.37, Figure 2).

Furthermore, the matched-pair analyses according to Del(Y) (194 cases per group, Supplemental Table 3) demonstrated that there was no significant difference in survival outcomes between the Del(Y) and Y-present groups (5y-OS 42.7% vs 47.6%, P = 0.31, Figure 4a; 5y-NRM 23.0% vs 24.7%, P = 0.94, Figure 4b; 5y-CIR 36.2% vs 33.2%, P = 0.48, Figure 4c). The cumulative incidences of acute and chronic GVHD were also equivalent between the Del(Y) and Y-present groups (5y-grade II - IV acute GVHD 38.9% vs 42.8%, P = 0.38; 5y-chronic GVHD 34.0% vs 39.1%, P = 0.73).

Additionally, we checked their clinical outcomes according to sex mismatch separately in the Y-present and Del(Y) cohorts (Supplemental Figure 2). In the Y-present cohort, female-to-male allo-HCT was significantly associated with inferior OS (HR 1.08 [95% CI: 1.02 – 1.15], P = 0.0073), NRM (HR 1.09 [95% CI: 1.01 – 1.19], P = 0.032), and an increased risk of chronic GVHD (HR 1.16 [95% CI: 1.08 – 1.25], P <0.001). On the other hand, in the Del(Y) cohort, female-to-male allo-HCT was not significantly associated with any clinical outcomes.

**Discussion**

The current study evaluated the clinical significance of Del(Y) in male allo-HCT recipients. In the analysis of female-to-male allo-HCT recipients, Del(Y) was significantly associated with an
increased risk of relapse, and this adverse impact of Del(Y) on relapse was also confirmed in a matched-pair analysis. While Del(Y) was also significantly associated with inferior OS in a multivariate analysis, this tendency was not confirmed in the matched-pair analysis. On the other hand, Del(Y) was not significantly associated with any clinical outcomes in the cohort of male-to-male allo-HCT.

H-Y antigens, which are encoded by Y-chromosome, are considered to be important targets of the GVHD and GVL effect, since female donor cells without Y-chromosome theoretically recognize male tissues / cells which usually harbor Y-chromosome. In fact, previous studies have reported the presence of minor histocompatibility antigens including H-Y antigens in hematological tumor cells. Additionally, the presence of H-Y antigen-specific B cell and H-Y antibodies in sex-mismatched allo-HCT has also been reported previously. The incidence of chronic GVHD was higher than that in the other gender combination and in recipients with H-Y antibodies. Conversely, lack of H-Y antigen might lead to attenuation of the GVHD and GVL effect, and an attenuated GVL effect would induce a high incidence of relapse. This hypothesis seems to be consistent with the results of the current study. The lack of a reduced incidence of GVHD in this study might be explained by the tissue distribution of Del(Y). Generally, Del(Y) is considered to present mainly in blood cells, and most studies on Del(Y) used blood samples. Since there is limited information on Del(Y) in peripheral tissues other than blood cells, donor cells are likely to be sensitized by H-Y antigens and Del(Y) might have only limited impact on the incidence of GVHD. However, several studies reported that Del(Y) was found in tissues besides blood cells, such as buccal mucosa and brain. Therefore, it is possible that peripheral tissues other than blood cells
may acquire Del(Y) and this might contribute to modification of the GVHD mechanism and the
distribution of involved organs in allo-HCT recipients with Del(Y). However, further investigation is
essential to clarify this hypothesis.

Another explanation for the higher incidence of relapse in the Del(Y) group is the effect of aging or
additional chromosomal abnormalities. Aging is an important risk factor for Del(Y); the incidence of
Del(Y) was reported to be only 0.05% in subjects up to 15 years of age, and 1.34% in those aged 76 – 80 years. Aging seems to be associated with additional chromosomal abnormalities
such as complex karyotype. In this study, the Del(Y) group included more recipients with very high
DRI than the Y-present group, and this may be due to the high frequency of complex karyotype in the Del(Y) group. In addition, the impact of Del(Y) itself should be considered. Several studies have reported a relationship between Del(Y) and cancer risk including both its incidence and mortality
31-34. However, the relationship was not consistent among different cancer types 33,35,36. In terms of
hematological malignancies, Del(Y) was associated with a higher incidence and lower leukemic
transformation of MDS 37,38. A high frequency of Del(Y) cells was also observed in AML and MPN
patients 39. Moreover, a previous study reported a relationship between Del(Y) and clonal
hematopoiesis 40. Although large cohort studies did not reveal a significant relationship between
Del(Y) and hematological malignancies 33,35, Del(Y) might impact the mechanism of the advanced
progression for hematological malignancies. However, the adverse impact of Del(Y) in
female-to-male allo-HCT was confirmed even after the effects of higher DRI such as additional
chromosomal abnormalities and older age were adjusted by a multivariate Cox model and
matched-pair analysis. On the other hand, the impact of Del(Y) on relapse was not observed in the
male-to-male allo-HCT cohort. These results suggest that the impact of Del(Y) on relapse in female-to-male allo-HCT may be due to loss of the GVL effect of H-Y alloreaction and that additional chromosomal abnormalities or Del(Y) itself have very limited impact on the results of CIR.

We initially expected that Del(Y) might be associated with some post-transplantation complications, since Del(Y) is related to several age-related diseases such as cardiovascular disease, Alzheimer disease, and diabetes $^{41,42}$. However, NRM and causes of non-relapse death were comparable between the Del(Y) and Y-present groups. Therefore, the clinical impact of Del(Y) on post-transplant complications was considered to be limited.

This study has several limitations due to its retrospective nature. First, the timing of the acquisition of karyotype information was heterogeneous. The registry data system we used did not collect karyotype information at the transplantation except for MDS patients, and most karyotype information was collected at the diagnosis. In some patients, the leukemic karyotype may have changed before transplantation following several chemotherapies or relapse events, and the impact of Del(Y) might be underestimated. Second, since information on the number of cells with Del(Y) could not be extracted from this registry data, the impact of Del(Y) might be somewhat inaccurate and overestimated. Therefore, a quantitative method should be considered in further studies $^{28,32,43}$. Third, there were only a limited number of Del(Y) patients. Thus, subgroup analyses stratified according to disease type and donor type could not be performed due to the small sample size. We applied matched-pair analyses in addition to multivariate analyses, for the purpose of adjusting the heterogeneity in the study cohort as far as possible. However, information on gene
mutations was not available in the current registry data-based study. Despite these limitations, to
the best of our knowledge, this is the first study to demonstrate the clinical significance of Del(Y) in
allo-HCT.

In conclusion, Del(Y) was significantly associated with inferior CIR. This result was confirmed by
multivariate Cox and matched-pair analyses. A higher incidence of relapse might be caused by
attenuation of the GVL effect due to a lack of H-Y antigens. Since a GVL effect by a sex-mismatch
itself may not be expected in male recipients with Del(Y) who receive allo-HCT from a female donor,
additional post-HCT strategies might be required to prevent disease relapse, such as consolidation
/maintenance therapy. Further studies are warranted to validate the results of this study.
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Author contributions:

M.Tamaki and H.N. conceived the original idea. M.Tamaki designed the study, analyzed data, and wrote the manuscript. K.K., SI.K., N.H and K.Y. advised on methods and wrote the manuscript. N.U., N.D., M.Tanaka, K.I., M.S., Y.Katayama, S.M., T.A., and Y.Kanda collected data and revised the manuscript. J.K., M.O., and T.F. collected data, revised the manuscript, and were responsible for data management at JSTCT. Y.A. managed the unified registry database and revised the manuscript. H.N. designed the study, advised on the methods, analyzed data, wrote and revised the manuscript, and was responsible for this project of JSTCT Transplant Complications Working Group. All authors approved the final version of the manuscript.

Conflict-of-interest disclosure:

SI.K. received honoraria from MSD, Sumitomo Dainippon Pharma, Pfizer, Astellas, Kyowa Kirin, Chugai Pharmaceutical, Bristol-Myers Squibb, Ono Pharmaceutical, Eisai, Nippon Kayaku, Takeda Pharmaceutical and SymBio Pharmaceuticals. J.K. has consulted for Astellas Pharma, SymBio Pharmaceuticals, Takeda Pharmaceutical, Megakaryon, Janssen Pharmaceutical and Daiichi
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Table 1. Patient characteristics of female-to-male patients.

|                        | Del(Y) (n = 155) | Y-present (n = 4149) | P value  |
|------------------------|------------------|---------------------|----------|
| **Age, n (%)**         |                  |                     |          |
| ≤ 50 years             | 58 (37.4)        | 1740 (41.9)         | 0.28     |
| > 50 years             | 97 (62.6)        | 2409 (58.1)         |          |
| **Disease type, n (%)**|                  |                     |          |
| AML                    | 116 (74.8)       | 2350 (56.6)         | <0.001   |
| ALL                    | 15 (9.7)         | 855 (20.6)          |          |
| MDS                    | 24 (15.5)        | 790 (19.0)          |          |
| MPN                    | 0 (0.0)          | 154 (3.7)           |          |
| **DRI, n (%)**         |                  |                     |          |
| Low                    | 25 (16.1)        | 139 (3.4)           | <0.001   |
| Intermediate           | 44 (28.4)        | 2379 (57.3)         |          |
| High                   | 45 (29.0)        | 1363 (32.9)         |          |
| Very high              | 41 (26.5)        | 268 (6.5)           |          |
| **HCT-CI, n (%)**      |                  |                     |          |
| 0 – 1                  | 90 (58.1)        | 2964 (71.4)         | <0.001   |
| ≥ 2                    | 64 (41.3)        | 1151 (27.7)         |          |
| **PS, n (%)**          |                  |                     |          |
| 0 – 1                  | 135 (87.1)       | 3773 (90.9)         | 0.11     |
| 2 – 4                  | 20 (12.9)        | 369 (8.9)           |          |
| **Donor type, n (%)**  |                  |                     |          |
| Matched related        | 38 (24.5)        | 982 (23.7)          | 0.93     |
| Matched unrelated      | 24 (15.5)        | 713 (17.2)          |          |
| Mismatched related     | 14 (9.0)         | 432 (10.4)          |          |
| Mismatched             | 73 (47.1)        | 1916 (46.2)         |          |
| **Donor source, n (%)**|                  |                     |          |
| Bone marrow            | 42 (27.1)        | 1375 (33.1)         | 0.44     |
| Peripheral blood       | 49 (31.6)        | 1207 (29.1)         |          |
| Cord blood             | 64 (41.3)        | 1559 (37.6)         |          |
| **Conditioning regimen, n (%)** |          |                     |          |
| MAC                    | 100 (64.5)       | 2835 (68.3)         | 0.33     |
| RIC                    | 55 (35.5)        | 1313 (31.6)         |          |
| **GVHD prophylaxis, n (%)** |            |                     |          |
| CsA-based              | 38 (24.5)        | 1251 (30.2)         | 0.21     |
| TAC-based              | 113 (72.9)       | 2826 (68.1)         |          |
| Other                  | 4 (2.6)          | 71 (1.7)            |          |
| **T cell in vivo depletion, n (%)** |         |                     |          |
| +                      | 14 (9.0)         | 443 (10.7)          | 0.60     |
| -                      | 141 (91.0)       | 3706 (89.3)         |          |

Abbreviations: Del(Y), deletion of Y-chromosome; Y-present, presence of Y-chromosome; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; DRI, disease risk index; HCT-CI, hematopoietic cell transplantation comorbidity index; PS, performance status; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; TAC, tacrolimus.
Table 2. Multivariate analyses of allo-HCT outcomes in female-to-male transplant recipients.

|                  | OS HR (95% CI) | P value | NRM HR (95% CI) | P value | Relapse HR (95% CI) | P value |
|------------------|----------------|---------|-----------------|---------|--------------------|---------|
| Del(Y)           | 1.25 (1.01 - 1.54) | 0.042   | 1.09 (0.778 - 1.52) | 0.62    | 1.40 (1.08 - 1.80) | 0.010   |
| Age > 50 years   | 1.70 (1.54 - 1.88) | <0.001  | 2.08 (1.80 - 2.41) | <0.001  | 1.19 (1.05 - 1.36) | 0.0090  |
| Disease          |                |         |                 |         |                    |         |
| AML              | Reference      | 1.0     | Reference       | 1.0     | Reference          | 1.0     |
| ALL              | 0.980 (0.864 - 1.11) | 0.75    | 1.01 (0.849 - 1.21) | 0.90    | 1.04 (0.884 - 1.23) | 0.61    |
| MDS              | 0.880 (0.787 - 0.924) | 0.024   | 1.03 (0.881 - 1.20) | 0.74    | 0.750 (0.641 - 0.876) | <0.001  |
| MPN              | 1.24 (1.01 - 1.54) | 0.045   | 1.50 (1.13 - 1.99) | 0.0047  | 1.21 (0.901 - 1.63) | 0.20    |
| DRI              |                |         |                 |         |                    |         |
| Low              | Reference      | 1.0     | Reference       | 1.0     | Reference          | 1.0     |
| Intermediate     | 1.33 (1.00 - 1.76) | 0.0049  | 1.23 (0.857 - 1.77) | 0.26    | 1.39 (0.921 - 2.10) | 0.12    |
| High             | 2.67 (2.02 - 3.54) | <0.001  | 1.89 (1.31 - 2.72) | <0.001  | 4.03 (2.68 - 6.05) | <0.001  |
| Very high        | 4.50 (3.33 - 6.06) | <0.001  | 2.38 (1.56 - 3.61) | <0.001  | 7.71 (5.04 - 11.8) | <0.001  |
| HCT-CI ≥ 2       | 1.15 (1.05 - 1.27) | 0.0023  | 1.30 (1.15 - 1.48) | <0.001  | 0.955 (0.841 - 1.09) | 0.48    |
| PS 2 – 4         | 1.92 (1.69 - 2.19) | <0.001  | 1.95 (1.61 - 2.36) | <0.001  | 1.64 (1.38 - 1.95) | <0.001  |
| Donor type       |                |         |                 |         |                    |         |
| Matched related  | Reference      | 1.0     | Reference       | 1.0     | Reference          | 1.0     |
| Matched unrelated| 1.37 (1.14 - 1.63) | <0.001  | 1.42 (1.09 - 1.83) | 0.0083  | 1.03 (0.813 - 1.30) | 0.82    |
| Mismatched       | 1.35 (1.13 - 1.61) | 0.0011  | 1.64 (1.27 - 2.12) | <0.001  | 0.891 (0.704 - 1.13) | 0.33    |
| Mismatched       | 1.34 (1.09 - 1.64) | 0.0046  | 1.71 (1.29 - 2.26) | <0.001  | 0.850 (0.650 - 1.11) | 0.23    |
| Donor source     |                |         |                 |         |                    |         |
| Bone marrow      | Reference      | 1.0     | Reference       | 1.0     | Reference          | 1.0     |
| Peripheral blood | 1.15 (0.988 - 1.34) | 0.072   | 1.12 (0.900 - 1.40) | 0.31    | 1.08 (0.890 - 1.32) | 0.42    |
| Cord blood       | 1.06 (0.913 - 1.22) | 0.47    | 0.935 (0.769 - 1.14) | 0.50    | 1.15 (0.938 - 1.40) | 0.18    |
| Myeloablative conditioning | 1.13 (1.03 - 1.25) | 0.028 | 1.14 (0.998 - 1.31) | 0.054 | 0.974 (0.855 - 1.11) | 0.69 |
| GVHD             | CsA-based      | Reference | 1.0 | Reference | 1.0 | Reference | 1.0 |
| prophylaxis      | TAC-based      | 0.846 (0.758 - 0.927) | 0.0027 | 0.787 (0.676 - 0.917) | 0.0021 | 0.945 (0.814 - 1.10) | 0.45 |
| Others           | 1.05 (0.747 - 1.47) | 0.78    | 1.12 (0.701 - 1.79) | 0.63    | 1.19 (0.772 - 1.84) | 0.43    |
| T cell in vivo depletion | 1.09 (0.940 - 1.26) | 0.26 | 1.06 (0.858 - 1.31) | 0.58 | 1.20 (0.987 - 1.45) | 0.067 |

474 Abbreviations: allo-HCT, allogeneic hematopoietic cell transplantation; NRM, non-relapse mortality; OS, overall survival; HR, hazard ratio; CI, confidence interval; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; DRI, disease risk index; HCT-CI, hematopoietic cell transplantation comorbidity index; PS, performance status; GVHD,
graft-versus-host disease; CsA, cyclosporine; TAC, tacrolimus; Del(Y), deletion of Y-chromosome.
**Figure legends.**

Figure 1. Clinical outcomes of allogeneic hematopoietic cell transplantation from female donors to male recipients (female-to-male allo-HCT) in a univariate analysis. (a) Overall survival. (b) Non-relapse mortality. (c) Cumulative incidence of relapse.

Figure 2. Impact of deletion of Y-chromosome (Del(Y)) on clinical outcomes allogeneic hematopoietic cell transplantation from female donors to male recipients (female-to-male allo-HCT).

Figure 3. Clinical outcomes of allogeneic hematopoietic cell transplantation from female donors to male recipients (female-to-male allo-HCT) in a matched-pair cohort. (a) Overall survival. (b) Non-relapse mortality. (c) Cumulative incidence of relapse.

Figure 4. Clinical outcomes of allogeneic hematopoietic cell transplantation from male donors to male recipients (male-to-male allo-HCT) (a) Overall survival. (b) Non-relapse mortality. (c) Cumulative incidence of relapse.
Figure 1. Survival outcomes according to Del(Y).

(a) OS

(b) NRM

P = 0.64

(c) Relapse

P < 0.001
Figure 2. Impact of Del(Y) on survival outcomes.

| Clinical Outcomes | HR of Del (Y) | 95%-CI       | P value |
|-------------------|---------------|--------------|---------|
|                   | Female-to-male|              |         |
| OS                |               | 1.25         | [1.01 – 1.154] | 0.042 |
| NRM               |               | 1.09         | [1.09 – 1.52] | 0.62  |
| Relapse           |               | 1.40         | [1.08 – 1.80] | 0.010 |
|                   | Male-to-male  |              |         |
| OS                |               | 1.15         | [0.954 – 1.38] | 0.15  |
| NRM               |               | 1.08         | [0.824 – 1.43] | 0.56  |
| Relapse           |               | 1.17         | [0.925 – 1.49] | 0.19  |
Figure 3. Matched-pair analysis of female-to-male allo-HCT.

(a) OS

(b) NRM

(c) Relapse

\( P = 0.14 \)

\( P = 0.67 \)

\( P = 0.037 \)
Figure 4. Matched-pair analysis of male-to-male allo-HCT.

(a) OS

(b) NRM

(c) Relapse

|          | Number at risk | Months | Probability |        | Cumulative incidence |        |        | Cumulative incidence |        |
|----------|----------------|--------|-------------|--------|----------------------|--------|--------|----------------------|--------|
| Y-present| 194            | 83     | 47          | 31     | 17                   | 0.0    | 0.2    | 0.2                  | 0.4    |
| Del(Y)   | 194            | 68     | 43          | 20     | 13                   | 0.0    | 0.2    | 0.2                  | 0.4    |
| Y-present| 189            | 76     | 43          | 29     | 16                   | 0.2    | 0.4    | 0.4                  | 0.6    |
| Del(Y)   | 187            | 61     | 39          | 17     | 11                   | 0.2    | 0.4    | 0.4                  | 0.6    |

P = 0.31
P = 0.94
P = 0.48