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Highlights	·Donor *KIR2DS1* was associated with reduced risk of relapse after HLA-haploidentical allogeneic hematopoietic cell transplantation (allo-HCT) using post-transplantation cyclophosphamide (PT/Cy-haplo). ·Donor *KIR2DS1* was associated with improved overall survival with PT/Cy-haplo. ·GVL effect via NK cell alloreactivity was exerted in CR, but not in non-CR. ·Donor *KIR* genotyping and disease status should be assessed for donor selection. ·Elucidating mechanisms involved could lead to novel strategies for relapse therapy.
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Running Head: Donor *KIR2DS1*-mediated Decreased Relapse in CR at PT/Cy-haplo

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ABSTRACT

HLA-haploidentical allogeneic hematopoietic cell transplantation (allo-HCT) using post-transplantation cyclophosphamide (PT/Cy-haplo) is becoming the standard of care for patients without HLA-matched related or unrelated donors. PT/Cy-haplo could provide more patients the opportunity to receive allo-HCT, since most patients have more than one available HLA-haploidentical related donor candidate. In PT/Cy-haplo settings, however, an optimal donor selection algorithm has not yet been established. To contribute to the establishment of a donor selection formula based on disease status and killer-cell immunoglobulin-like receptor (KIR) genotype, we retrospectively analyzed 91 patients who received PT/Cy-haplo at our institute. In both patients and donors, HLA allele genotyping was performed for *HLA-A, -B, -C*, and -*DRB1* and 16 KIR genes were genotyped. Patients in complete remission (CR) who underwent PT/Cy-haplo from *KIR2DS1*-positive donors had significantly lower rates of cumulative incidence of relapse (CIR) than those who underwent PT/Cy-haplo from *KIR2DS1*-negative donors (1-year CIR, 0.0% vs. 32.6%, $P = 0.037$; 2-year CIR, 9.2% vs. 42%, $P = 0.037$). Moreover, PT/Cy-haplo from *KIR2DS1*-positive donors was significantly associated with improved overall survival (OS) (1-year OS, 91.7% vs. 58.7%, $P = 0.010$; 2-year OS, 83% vs. 34%, $P = 0.010$). In contrast, in non-CR individuals, PT/Cy-haplo from *KIR2DS1*-positive

donors did not significantly improve CIR or OS (1-year CIR 56.5% vs. 64.7%, *P* = 0.973; 2-year CIR, not reached vs. 64.7%, not evaluable; 1-year OS, 25.4% vs. 20.6%, *P* = 0.418; 2-year OS, 5.1% vs. 20.6%, *P* = 0.418). Additionally, lower infused $CD34^+$ cell dose, female-to-male transplantation, and acute myeloid leukemia were significantly associated with increased risk of relapse and mortality. In conclusion, the present study demonstrated that graft-versus-leukemia/tumor effects were exerted through donor *KIR2DS1* at PT/Cy-haplo when patients have low tumor burdens. It would be worth examining the inclusion of donor KIR genotyping and disease status assessment in establishing optimal donor selection criteria at PT/Cy-haplo.

Keywords: killer-cell immunoglobulin-like receptor (KIR) genotyping; *KIR2DS1*; HLA-haploidentical allogeneic hematopoietic cell transplantation using post-transplantation cyclophosphamide; complete remission; relapse; prognosis; survival

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative treatment for patients suffering from hematologic malignancies with poor prognosis. The lack of a conventional HLA-matched related or unrelated donor has been a serious barrier to allo-HCT. Increasing evidence indicates that T-cell-replete HLA-haploidentical allo-HCT using post-transplantation cyclophosphamide (PT/Cy-haplo) could become a standard mode of care due to its effectiveness and safety [1]. Therefore, PT/Cy-haplo could provide more patients the opportunity to receive allo-HCT, as most patients have more than one available HLA-haploidentical related donor candidate, including not only siblings, but also parents and children. Clinicians should, thus, aim to select the best donor to decrease the risk of relapse and improve prognosis when multiple donors are available. Therefore, it is warranted to establish an algorithm for optimal donor selection in PT/Cy-haplo settings.

Natural killer (NK) cell alloreactivity plays a crucial role in clinical outcomes of patients receiving T-cell-depleted haploidentical allo-HCT [2,3]. Alloreactivity is regulated by the integral balance between inhibitory and activating signals through cell-surface receptors, especially killer-cell immunoglobulin-like receptors (KIRs) [4,5]. Regarding donor selection in PT/Cy-haplo, only a few studies have examined the impact of *KIR* genotypes

on patient outcome [5-7]. In these, KIR mismatches, i.e., KIR receptor-ligand mismatches, the *KIR* B/x haplotype with *KIR2DS2*, and inhibitory KIR gene mismatches, were reported to be associated with lower rates of relapse and better survival [5-7]. However, the results remain inconclusive. Moreover, the impact of donor *KIR2DS1* positivity for prevention of relapse has never been investigated in PT/Cy-haplo studies, although it was reported previously in unrelated allo-HCT [8].

In addition, it is controversial how the graft-versus-leukemia/tumor (GVL) effects of *KIRs* or *HLAs* are modified by the residual tumor burden in haploidentical transplantation. NK alloreactive donors prevented patients in complete remission (CR) from relapsing leukemia, but not patients in non-CR (NCR) [3], whereas KIR ligand incompatibility was reported to be more beneficial to NCR patients [9].

We, therefore, retrospectively examined whether the remission status at PT/Cy-haplo modifies GVL effects by NK-cell alloreactivity through *KIR*, with the aim to contribute to the establishment of a donor selection formula based on disease status and donor KIR gene information.

MATERIALS AND METHODS

Patients

We retrospectively analyzed consecutive patients who received PT/Cy-haplo at our institute between June 2009 and December 2018. The study was approved by the Human Subjects Review Committee at Osaka City University (Osaka, Japan). Written informed consent from patients and donors was obtained in cases where blood samples were prospectively collected from July 2013 onward. Otherwise, we provided the opportunity to withdraw from the present study at any time for eligible living patients and donors. Furthermore, the study information was officially disclosed to the public on the website of the Department of Hematology, Graduate School of Medicine, Osaka City University, according to the Declaration of Helsinki and the Ethical Guidelines for Human Genome/Gene Analysis Research established by the Ministry of Health, Labour, and Welfare in Japan.

Transplantation Procedures

Detailed transplantation procedures used at our institute have been reported previously [10-12]. Briefly, granulocyte colony-stimulating factor-mobilized T-cell-replete peripheral blood grafts were infused on day 0 in all patients. The intravenous busulfan

(i.v. Bu)-based conditioning regimen consisted of 15 mg/m^2 fludarabine and 2,000 mg/m^2 cytarabine twice a day on days -11 and -10, 2.0 mg/kg rabbit anti-thymocyte globulin (rATG) once a day on days -8 and -7, 30 mg/m^2 fludarabine once a day on days -6 to -3, and 0.8 mg/kg i.v. Bu four times a day on days -6 to -3. The melphalan (Mel)-based conditioning regimen replaced i.v. Bu with 100 mg/m^2 Mel once a day on day -2. The refined Mel-based conditioning regimen skipped rATG administration since July 2013. Graft-versus-host disease (GVHD) prophylaxis consisted of 25 mg/kg PT/Cy once a day on days +3 and +4, tacrolimus that was infused continuously at a targeted blood concentration of 10–15 ng/mL from day +5, and oral mycophenolate mofetil from day +5. If GVHD did not occur, mycophenolate mofetil was discontinued at day +40 and tacrolimus was initiated to taper on the interval between day +60 and +100 and discontinued by day +180, if possible. Otherwise, patients were treated according to previously published procedures [5,13-15].

HLA and KIR Typing and KIR Mismatch Models

In both patients and donors, *HLA* allele typing was performed at *HLA-A, -B, -C*, and *-DRB1*, and 16 KIR genes were genotyped using the KIR SSO Genotyping Test (One Lambda, Inc., Canoga Park, CA, USA) according to the following procedures [16]: the

purified DNA extract was mixed with the PCR mixture, including primers for exons 3 + 4, 5, and 7–9, and amplified. Next, each PCR product was hybridized with beads, and sample test data were acquired using a LABScan^{TM} 3D System (One Lambda). The flow analyzer measured the fluorescent signal from R-phycoerythrin-conjugated streptavidin bound to biotinylated DNA for positive probe-beads in a reaction mixture. *KIR* genotyping was determined using an algorithm from the patterns of probe-bead reactions. Independent of collecting clinical data at our institute, *KIR* genotyping was performed at BML, INC. (Saitama, Japan).

According to KIR gene-gene models, each patient and donor was classified into either A/A or B/x group, and inhibitory KIR gene mismatched pairs were defined as previously reported [5,7,17,18]. The *KIR* A/A haplotype uniformly consists of *KIR3DL3, KIR2DL3, KIR2DP1, KIR2DL1, KIR3DP1, KIR2DL4, KIR3DL1, KIR2DS4*, and *KIR3DL2*; B/x haplotypes include one or more of the following genes: *KIR2DS2, KIR2DL2, KIR2DL5, KIR2DS3, KIR3DS1, KIR2DS5*, and *KIR2DS1*. In a KIR ligand incompatibility model, patients and donors were categorized into a combination of *C1/C1, C1/C2*, or *C2/C2* of *HLA-C* epitopes and *Bw4/Bw4, Bw4/Bw6*, or *Bw6/Bw6* of *HLA-B* epitopes, and ligand mismatched pairs were identified according to previous reports [9,19]. In the receptor-ligand model, combinations of donor *KIR* genotypes and patient KIR ligands

were categorized into either matched pairs or mismatched pairs via *KIR2DL1, KIR2DL2/3*, or *KIR3DL1* signaling pathways [5,6]. In the missing ligand model, mismatched patients had *C1/C1, C2/C2*, or *Bw6/Bw6* [3].

Definitions

All patients were classified according to the hematopoietic cell transplant-comorbidity index (HCT-CI) [20]. Acute GVHD was classified based on the standard grading system, and grades II to IV were considered to be clinically significant [21].

Disease status at PT/Cy-haplo was determined in this study according to the following methods: CR for acute myeloid leukemia (AML) patients was defined as both CR and CR with incomplete hematologic recovery, according to European LeukemiaNet recommendations 2017 [22]; CR for myelodysplastic syndrome patients was defined as both CR and marrow CR with respect to the International Working Group response criteria in myelodysplasia 2006 [23]; no one was considered to be in CR in chronic myelogenous leukemia patients; CR for acute lymphoblastic leukemia was defined according to a previous study [24]; CR for non-Hodgkin lymphoma, including adult T-cell leukemia/lymphoma, was defined according to The Lugano Classification 2014 or the Response Criteria of Adult T-cell Leukemia/Lymphoma International Consensus Meeting

2009 [25, 26]. Unless in CR, remaining patients were defined as NCR.

Relapse for the CR population was defined as morphological or pathological recurrence of primary disease, and relapse for the NCR population was defined as the first point to detect morphological or pathological evidence of recurrence/progression of primary disease after PT/Cy-haplo [27].

Statistical Analysis

Cumulative incidence of relapse (CIR) was estimated using a cumulative incidence curve with non-relapse mortality (NRM) as a competing risk and compared using Gray's test. Moreover, death without acute GVHD, relapse, and subsequent transplantation, were considered to be competing risks for acute GVHD [28]. Overall survival (OS) was estimated by Kaplan-Meier curve plots and compared statistically using the log-rank test. Subsequent transplantation was considered as an event instead of censoring for OS according to the European Society for Blood and Marrow Transplantation Statistical Guidelines [29]. In univariable and multivariable analyses of prognostic factors, a Cox proportional hazards model was used for OS, and a cause-specific hazard model was used for relapse and NRM [30]. The proportionality of hazards assumption was evaluated using scaled Schoenfeld residuals and log-minus-log plots. Non-linear effects

of continuous independent variables were tested using log transformations [31]. As an additional analysis, the Fine-Gray sub-distribution hazard model was used to assess prognostic factors for relapse.

All *P* values were two sided, and a *P* value < 0.05 was considered to be statistically significant. All confidence intervals (CIs) were 95%. Statistical analyses were performed using EZR version 1.35 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [32] and SPSS Statistics 24 (IBM, Armonk, NY, USA).

RESULTS

Patient Characteristics

A total of 91 patients with available *KIR* typing data were included in the study. Of these, *HLA* genotype data were unavailable for five patients (5.5%). Patient and donor characteristics are shown in Tables 1 and 2 and Supplementary Tables 1 and 2. The median patient age was 48 years (range, 17–68 years). The median follow-up period among survivors was 1,271 days (range, 242–3,135 days). Thirty-four patients (37%) exhibited CR at PT/Cy-haplo. In the CR and NCR groups, 12 (35%) and 23 patients (40%) received PT/Cy-haplo from *KIR2DS1*-positive donors, respectively. For patients for whom *HLA* data were available, 76 *HLA-C* mismatched transplants (88%) were

included (Supplementary Table 2), and the frequencies of donor KIR ligand were 78 (91%) for *C1/C1*, 8 (9%) for *C1/C2*, and 0 for *C2/C2* (Supplementary Table 1). The distribution of *KIR2DS1, KIR2DL5, KIR3DS1*, and *KIR2DS5* in donors was almost the same (Table 1 and 2). We focused on *KIR2DS1* in univariable and multivariable analyses, because it has been reported that *KIR2DS1* is in positive genetic linkage disequilibrium with these genes, and *KIR2DS1* is the best characterized activating *KIR* in a number of *in vitro* functional analyses [4,18,33,34]. This is also why the effect of *KIR2DS1* positivity on clinical outcomes after allo-HCT has been well-addressed previously [8,35,36].

Relapse and Overall Survival Associated with Donor KIR Positivity According to Remission Status at PT/Cy-haplo

In the CR population at PT/Cy-haplo, patients who underwent PT/Cy-haplo from *KIR2DS1*-positive donors had significantly lower rates of CIR than those who underwent PT/Cy-haplo from *KIR2DS1*-negative donors (2-year CIR, 9.2% vs. 42%; $P = 0.037$; Figure 1). We were unable to perform subgroup analysis using information regarding the *HLA-C* epitope *C1* or *C2* status of the donors and patients, because there were no *C2*/*C2*-recipients, similar to a previous report [37], and all three *C1*/*C2*-recipients in CR received PT/Cy-haplo from *KIR2DS1*-positive donors and the two *C1*/*C2*-recipients in

NCR received PT/Cy-haplo from *KIR2DS1*-negative donors (Supplementary Table 1). In PT/Cy-haplo from a *KIR2DL5*- or *KIR3DS1*-positive donor, similar results were obtained, most likely due to genetic linkage disequilibrium among these genes. No other positivities of donor KIR genes were associated with CIR. Furthermore, PT/Cy-haplo from a *KIR2DS1*-positive donor was significantly associated with improved OS (2-year OS, 83% vs. 34%; $P = 0.01$; Figure 1E). Moreover, PT/Cy-haplo from a B/x donor significantly increased OS (2-year OS, 77% vs. 35%, $P = 0.019$), but did not decrease CIR (2-year CIR, 16% vs. 40%, $P = 0.122$; Supplementary Figure 1).

In the NCR population, however, PT/Cy-haplo from a *KIR2DS1*-positive donor and a B/x donor did not significantly improve CIR or OS (Figures 1B and 1F). Furthermore, donor *KIR2DS1* positivity was not statistically associated with cumulative incidence of acute GVHD regardless of CR or NCR (Figures 1G and 1H).

Univariable and Multivariable Analyses for Relapse, NRM, and OS

In univariable and multivariable analyses, the insertion of log transformations of all continuous variables except infused $CD34^+$ cell dose (median 4.5, range 2.1–37.8 [$\times 10^6$/kg]) into all models did not result in an improved fit compared with the linear model. As only infused $CD34^+$ cell dose showed a non-linear association with risk of relapse in

all models, we fitted a model using infused $CD34^+$ cell dose categorized into tertiles (2.1–3.7, 3.8–5.6, and 5.7–37.8 [$\times 10^6$/kg]) to account for this non-linearity [31].

Detailed univariable analysis is shown in Table 3. In the CR population, PT/Cy-haplo from *KIR2DS1*-positive donor was significantly associated with decreased risk of relapse and OS. In addition, it was not associated with increased risk of NRM. Administration of rATG was statistically significantly associated with increased risks of relapse and mortality. Moreover, high scores of HCT-CI and older donors were related to increased risk of NRM, and high HCT-CI scores and number of allo-HCTs were related to increased risk of OS.

On the contrary, in the NCR population, PT/Cy-haplo from *KIR2DS1*-positive donors was not associated with a decreased risk of relapse and OS, but rather, it was associated with an increased risk of NRM. In addition, male patients, AML cases, female-to-male transplantation, and low levels of infused $CD34^+$ cells were related to increased risk of relapse, and male patients were at increased risk of NRM. Furthermore, male patients, AML cases, female-to-male transplantation, and low levels of infused $CD34^+$ cells were related to increased risk of OS.

We constructed several multivariable models involving donor *KIR2DS1* positivity to limit the degrees of freedom of the models from the viewpoint of stability (Table 4) [31]. In

models 1, 2, and 3, PT/Cy-haplo from *KIR2DS1*-positive donors was significantly associated with improved risk of relapse and OS among CR patients. In models 4 and 5, PT/Cy-haplo from *KIR2DS1*-positive donors was significantly associated with a decreased risk of mortality. Administration of rATG was marginally significantly associated with increased risk of relapse and was significantly associated with poorer OS. In contrast, there were no models in which PT/Cy-haplo from a *KIR2DS1*-positive donor was associated with improved risk of relapse and OS among NCR patients. However, lower infused $CD34^+$ cell numbers, female-to-male transplantation, and presence of AML were associated with increased risk of relapse and mortality (Table 4, Figure 2, and Supplementary Figure 2).

Outcomes in Previously-reported Models

In our cohort, mismatched pairs in the receptor-ligand model completely coincided with mismatched pairs in the missing ligand model; thus, we employed the missing ligand model because we did not have to consider *KIR* genotypes in this model. Mismatched CR patients in the missing ligand model were associated with improved relapse and survival, although most of them had mismatches in this model (88%; Supplementary Tables 2 and 3). On the contrary, all of the following models were not

statistically significant in terms of relapse and survival: inhibitory KIR gene model, KIR ligand incompatibility model, and *HLA-DRB1* disparity in GVHD.

CMV reactivation by donor KIR2DS1

We assessed CMV reactivation incidence according to presence of donor *KIR2DS1* (Supplementary Figure 8). We did not observe any significant difference in CMV reactivation incidence by donor *KIR2DS1*.

DISCUSSION

We found that donor *KIR2DS1* positivity significantly contributed to decreased risk of both relapse and mortality in the CR population, but not in the NCR population at PT/Cy-haplo. GVL effects mediated by *KIR2DS1*-positive donors were observed when the residual tumor burden was low.

In previous PT/Cy-haplo studies using genotyping information regarding *KIR*s, the following factors associated with a lower rate of relapse have been reported: KIR receptor-ligand mismatch [6], *KIR* B/x haplotype with *KIR2DS2* [5], and inhibitory KIR gene mismatch [7]. Actually, the KIR receptor-ligand/missing ligand mismatch was associated with decreased risk of relapse and mortality in CR patients, but only a few of

our patients (9%) were matched in this model (Supplementary Table 3). Therefore, we could not conclude the significance of this model in the present study. In addition, donor *KIR* B/x with *KIR2DS2* was not associated with decreased risk of relapse (B/x with *KIR2DS2* vs. B/x without *KIR2DS2*: HR in CR patients 1.9, 95% CI 0.1-31.3, $P = 0.643$; HR in NCR patients 0.8, 95% CI 0.2-2.7, $P = 0.683$). Although we could not clarify the reason for inconsistent results with the previous report by Solomon, these might be explained by differences in disease type, disease status, the profile of KIR genes based on ethnicity, and the dose of PT/Cy [5]. In our study, PT/Cy-haplo from a B/x donor significantly increased OS, but did not decrease CIR. These results suggested that prevention of leukemia/tumor relapse could be influenced by donor *KIR2DS1* status rather than donor B/x haplotype.

To the best of our knowledge, this is the first report in which both the GVL effect and improved survival using donor *KIR2DS1* have been demonstrated in PT/Cy-haplo. Several experimental studies have demonstrated that *KIR2DS1* plays a role in cell-killing effects of alloreactive NK cells against leukemia cells expressing HLA-C2 [17,38-42]. Recent data suggest that weak or non-inhibiting combinations of *KIR3DL1/HLA-B* subtypes have independent and additive effects in preventing leukemia relapse in unrelated allo-HCT from *KIR2DS1*-positive donors [43]. Further study is needed to clarify

the effect of *KIR3DL1/HLA-B* subtypes according to donor *KIR2DS1* status on the prevention of relapse in PT/Cy-haplo settings.

In the present study, GVL effects by NK alloreactivity was apparent only in the CR population. This is consistent with results of previous studies in which GVL effects induced by GVHD and donor lymphocyte infusion were observed only in the CR population [44-46]. Ruggeri's study supports our data [3], whereas Wanquet's report suggests that ligand mismatches are associated with reduced risks of relapse and progression-free survival only in the NCR population [9]. This discrepancy might result from the fact that Wanquet's study included more lymphoid diseases and the KIR ligand mismatch model is an estimated one using only *HLA* information without *KIR* genotyping. Russo et al. suggested that infused donor-derived mature NK cells would be eliminated by PT/Cy, probably leading to dampened NK alloreactivity in early phases of PT/Cy-haplo [47]. These data appear to support our finding that NK alloreactivity through donor *KIR2DS1* status was not observed in the NCR population because NK cell recovery would require more than at least 60 days post-PT/Cy-haplo [10], and this interval would bring disadvantage to groups with high tumor burden who require earlier exertion of GVL effects for controlling leukemia/tumor progression. Moreover, our PT/Cy-haplo protocols might result in higher numbers of NK cells because of the

relatively low dose of PT/Cy employed here compared with the originally-reported dose of PT/Cy [10-12]. From analyses of our previous data [10], we were unable to find any obvious difference in the numbers of NK cells as the marker of NK-cell reconstitution by the presence of *KIR2DS1* in the donors (Supplementary Figure 7). NK cell functions may be affected by the presence of *KIR2DS1* in donors, and a future study is warranted to explore this effect.

In this study, limited to the NCR population, the dose of infused $CD34^+$ cells and female-to-male transplantation significantly influenced the incidence of relapse. Although previous data on higher $CD34^+$ cell doses associated with improved survival might support this result [48], we could not identify the reason why female-to-male transplantation elevated the risk of relapse in the NCR population. Further study is required to confirm these findings. On the basis of these observations, the following strategy would be worth testing to prevent relapse in PT/Cy-haplo settings: *KIR2DS1*-positive donors should be selected for patients with low tumor burden, whereas patients with high tumor burden require other strategies; for example, on the basis of the donor/recipient gender and infused $CD34^+$ cell dose.

Stratified analyses by myeloid or lymphoid malignancies suggested that beneficial donor *KIR2DS1* effect may not be affected by myeloid or lymphoid malignancies in CR

population (Supplementary Figures 5 and 6). However, due to the small sample size, further large-scale study is needed to confirm this.

The interpretation of our results may be limited by the nature of a retrospective study conducted at a single center. However, since almost all patients had been enrolled in previous prospective trials [10-12], they had been managed homogeneously according to study protocols, leading to reduced bias. As per the study protocols [10-12], although almost all patients received either the busulfan-based conditioning or the melphalan-based conditioning, conditioning regimens were not associated with risk of relapse or OS (Table 3). Furthermore, there was heterogeneity in administration of rATG. However, the stratified analyses by rATG and the multivariable analyses appear to support that donor *KIR2DS1* was associated with a reduced risk of relapse regardless of rATG administration (Table 4, and Supplementary Figures 3 and 4). In addition, all patients, most of whom received 25 mg/kg PT/Cy for two days, were infused with peripheral blood grafts and administered lower doses of PT/Cy than the original PT/Cy dose, because we had designed the previous protocol under the hypothesis that the relatively high dose of PT/Cy could attenuate GVL effects [10-12]. However, these might contribute to eliciting GVL effects through NK alloreactivity more effectively.

In conclusion, the present study demonstrated that exertion of GVL effects by donor

KIR2DS1 required a low tumor burden at PT/Cy-haplo. In addition, our data suggested that a different strategy may be required in high tumor burden conditions in order to decrease the risk of relapse. It would be worth testing the inclusion of donor *KIR* genotyping and tumor burden evaluations for establishing optimal donor selection criteria at PT/Cy-haplo in the future.

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Conflict of interest statement: There are no conflicts of interest to disclose.

Table

Table 1. Patient Characteristics at HLA-Haploidentical Allogeneic Hematopoietic Cell Transplantation Using Post-transplantation Cyclophosphamide According to Remission Status

Characteristic, n (%)	CR Patients (n = 34)	NCR Patients (n = 57)
Median age (range), years	50 (21–67)	48 (17–68)
Sex, male	21 (62)	36 (63)
Disease		
AML	20 (59)	34 (60)
MDS	2 (6)	4 (7)
CML	0 (0)	2 (4)
ALL	7 (21)	6 (11)
NHL	5 (15)	11 (19)
HCT-CI		
0	19 (56)	19 (33)
1-2	10 (29)	19 (33)
≥ 3	5 (15)	19 (33)
Conditioning regimen		
Bu-based	6 (18)	14 (25)
Mel-based	28 (82)	40 (70)
Others	0 (0)	3 (5)
TBI	2 (6)	5 (9)
rATG	12 (35)	20 (35)
PT/Cy		
25	2 (6)	4 (7)
25–25	30 (88)	48 (84)
50–50	2 (6)	5 (9)
Immunosuppressant		
Tac	32 (94)	57 (100)
CsA	2 (6)	0 (0)
MMF	34 (100)	56 (98)
Number of allo-HCT		
1	22 (65)	32 (56)
2	11 (32)	20 (35)
3	1 (3)	5 (9)
Donor age, median (range) years	31 (15-62)	35 (12-66)
Female donor to male patient	7 (21)	16 (28)

ABO mismatch	14 (41)	22 (39)
Infused $CD34^+$ cell dose*, median (range) [$\times 10^6$/kg]	4.4 (2.2–19.7)	4.7 (2.1–37.8)
CMV serostatus (donor/recipient)		
+/+	20 (59)	44 (77)
+/-	2 (6)	1 (2)
-/+	10 (29)	10 (18)
-/-	2 (6)	1 (2)
unknown	0 (0)	1 (2)
HLA disparity		
4/8	20 (59)	30 (53)
5/8	11 (32)	19 (33)
6/8	1 (3)	4 (7)
7/8	1 (3)	0 (0)
unknown	1 (3)	4 (7)
Donor *KIR* genotype		
3DL3	34 (100)	57 (100)
2DS2	5 (15)	6 (11)
2DL2	5 (15)	6 (11)
2DL3	34 (100)	57 (100)
2DL5	12 (35)	23 (40)
2DS3	2 (6)	9 (16)
2DP1	34 (100)	57 (100)
2DL1	34 (100)	57 (100)
3DP1	34 (100)	57 (100)
2DL4	34 (100)	57 (100)
3DS1	12 (35)	22 (39)
3DL1	32 (94)	55 (96)
2DS5	11 (32)	16 (28)
2DS1	12 (35)	23 (40)
2DS4	32 (94)	55 (96)
3DL2	34 (100)	57 (100)

Abbreviations: ALL, acute lymphoblastic leukemia; allo-HCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; Bu, busulfan; CD, cluster of differentiation; CML, chronic myelogenous leukemia; CMV, cytomegalovirus; CR, complete remission; CsA,

cyclosporine; HCT-CI, hematopoietic cell transplant-comorbidity index; KIR, killer-cell immunoglobulin-like receptor; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; NCR, non-complete remission; NHL, non-Hodgkin lymphoma; Mel, melphalan; PT/Cy, post-transplantation cyclophosphamide; rATG, rabbit anti-thymocyte globulin; Tac, tacrolimus; TBI, total body irradiation

* The infused $CD34^+$ cell dose was analyzed in 31 CR patients and 53 NCR patients.

Table 2. KIR Genotyping of 91 Pairs of Donors and Recipients

Haplo-type	Geno-type ID	*3DL3*	*2DS2*	*2DL2*	*2DL3*	*2DL5*	*2DS3*	*2DP1*	*2DL1*	*3DP1*	*2DL4*	*3DS1*	*3DL1*	*2DS5*	*2DS1*	*2DS4*	*3DL2*	Donor frequency	Recipient frequency
AA	1	+	-	-	+	-	-	+	+	+	+	-	+	-	-	+	+	51 (56)	44 (48)
Bx	2	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	19 (21)	17 (19)
Bx	3	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	2 (2)	4 (4)
Bx	4	+	+	+	+	-	-	+	+	+	+	-	+	-	-	+	+	5 (5)	4 (4)
Bx	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1 (1)	0 (0)
Bx	7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	1 (1)	1 (1)
Bx	8	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	7 (8)	13 (14)
Bx	9	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	1 (1)	2 (2)
Bx	11	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	0 (0)	1 (1)
Bx	64	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	0 (0)	1 (1)
Bx	69	+	-	-	+	+	-	+	+	+	+	+	-	+	+	-	+	2 (2)	3 (3)
Bx	70	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	1 (1)	0 (0)
Bx	75	+	-	-	+	+	+	+	+	+	+	+	-	+	+	-	+	1 (1)	1 (1)

Table 3. Univariable Analyses of Relapse, Non-relapse Mortality, and Overall Survival in CR and NCR Patients

	Relapse		Non-relapse Mortality		Overall Survival	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
(A) CR Patients						
Patient age (per 10 years)	1.0 (0.6–1.5)	0.841	1.4 (0.8–2.4)	0.268	1.1 (0.8–1.6)	0.486
Sex, male	0.5 (0.2–1.9)	0.338	1.8 (0.4–9.0)	0.467	0.9 (0.3–2.3)	0.788
Disease						
AML	0.8 (0.2–3.0)	0.793	0.6 (0.1–2.4)	0.462	0.8 (0.3–2.0)	0.587
Non-AML	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
HCT-CI ≥ 3	2.5 (0.5–11.8)	0.258	5.7 (1.2–26.0)	0.025	3.7 (1.3–11)	0.017
Conditioning regimen						
Bu-based	1.3 (0.3–5.9)	0.778	0.8 (0.1–6.2)	0.804	1.1 (0.3–3.7)	0.902
Mel-based or others	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
rATG (vs. No rATG)	4.0 (1.1–14.3)	0.034	2.6 (0.6–10.8)	0.183	3.2 (1.2–8.1)	0.016
Number of allo-HCT						
1	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
2 or 3	2.7 (0.8–9.6)	0.117	2.8 (0.7–12.3)	0.163	2.7 (1.0–6.9)	0.040
Donor age (per 10 years)	0.9 (0.5–1.6)	0.743	1.7 (1.0–2.8)	0.049	1.2 (0.9–1.8)	0.249
Female donor-to-male patient	0.7 (0.2–3.5)	0.707	1.2 (0.2–5.8)	0.858	0.9 (0.3–2.6)	0.790
ABO mismatch	0.6 (0.1–2.2)	0.427	2.5 (0.6–10.4)	0.217	1.2 (0.5–3.0)	0.733
Infused $CD34^+$ cell dose [$\times 10^6$/kg]						
Tertile 1	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
Tertile 2	0.6 (0.1–2.5)	0.438	3.6 (0.4–30.8)	0.244	1.2 (0.4–3.6)	0.795
Tertile 3	0.4 (0.1–2.3)	0.322	1.0 (0.1–16.7)	0.975	0.6 (0.1–2.3)	0.426

CMV donor -/recipient +	1.1 (0.3–4.2)	0.905	1.5 (0.4–6.4)	0.571	1.4 (0.5–3.8)	0.470
HLA disparity 4/8	6.5 (0.8–51.7)	0.079	0.8 (0.2–3.3)	0.774	1.8 (0.6–5.0)	0.286
Donor *KIR* genotype						
2DS2	0.6 (0.1–4.6)	0.608	0.7 (0.1–5.9)	0.767	0.7 (0.2–3.1)	0.645
2DL2	0.6 (0.1–4.6)	0.608	0.7 (0.1–5.9)	0.767	0.7 (0.2–3.1)	0.645
2DL5	0.1 (0.0–0.9)	0.043	0.4 (0.1–2.2)	0.305	0.2 (0.1–0.8)	0.018
2DS3	NA	NA	1.6 (0.2–13.1)	0.667	0.7 (0.1–5.1)	0.698
3DS1	0.1 (0.0–0.9)	0.043	0.4 (0.1–2.2)	0.305	0.2 (0.1–0.8)	0.018
2DS5	0.1 (0.0–1.1)	0.065	0.5 (0.1–2.6)	0.420	0.3 (0.1–0.9)	0.040
2DS1	0.1 (0.0–0.9)	0.043	0.4 (0.1–2.2)	0.305	0.2 (0.1–0.8)	0.018
Donor *KIR* haplotype						
A/A	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
B/x	0.2 (0.1–1.1)	0.074	0.4 (0.1–1.8)	0.225	0.3 (0.1–0.9)	0.027
(B) NCR Patients						
Patient age (per 10 years)	1.0 (0.8–1.2)	0.836	1.2 (0.8–1.8)	0.344	1.1 (0.9–1.3)	0.590
Sex, male	2.1 (1.0–4.3)	0.041	5.0 (1.4–18.5)	0.016	2.3 (1.2–4.2)	0.009
Disease						
AML	2.1 (1.0–4.3)	0.040	1.3 (0.5–3.7)	0.626	1.9 (1.1–3.5)	0.033
Non-AML	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
HCT-CI ≥ 3	0.8 (0.4–1.7)	0.592	2.1 (0.7–5.7)	0.161	1.3 (0.7–2.3)	0.418
Conditioning regimen						
Bu-based	0.8 (0.4–1.8)	0.644	1.8 (0.6–5.5)	0.276	1.3 (0.7–2.5)	0.419
Mel-based or others	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
rATG (vs. No rATG)	1.1 (0.5–2.1)	0.827	0.9 (0.3–2.7)	0.869	1.0 (0.6–1.8)	0.922

Number of allo-HCT						
1	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
2 or 3	1.1 (0.6–2.2)	0.692	1.7 (0.6–4.8)	0.313	1.2 (0.7–2.2)	0.474
Donor age (per 10 years)	1.0 (0.8–1.2)	0.688	0.8 (0.5–1.2)	0.276	0.9 (0.7–1.1)	0.295
Female donor-to-male patient	2.8 (1.4–5.7)	0.004	1.5 (0.4–5.7)	0.523	1.9 (1.0–3.6)	0.036
ABO mismatch	0.9 (0.5–1.8)	0.746	1.7 (0.6–5.1)	0.342	1.1 (0.6–2.0)	0.676
Infused $CD34^+$ cell dose [$\times 10^6$/kg]						
Tertile 1	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
Tertile 2	0.4 (0.2–0.8)	0.016	0.2 (0.0–0.4)	0.113	0.4 (0.2–0.8)	0.010
Tertile 3	0.3 (0.1–0.8)	0.009	0.6 (0.2–2.4)	0.510	0.5 (0.3–1.0)	0.052
CMV donor-/recipient +	0.6 (0.2–1.6)	0.303	2.0 (0.7–5.8)	0.218	1.0 (0.5–2.0)	0.938
HLA disparity 4/8	1.1 (0.6–2.2)	0.764	1.9 (0.6–5.6)	0.271	1.5 (0.8–2.7)	0.203
Donor *KIR* genotype						
2DS2	0.8 (0.3–2.8)	0.780	2.3 (0.6–8.5)	0.206	1.3 (0.5–3.0)	0.571
2DL2	0.8 (0.3–2.8)	0.780	2.3 (0.6–8.5)	0.206	1.3 (0.5–3.0)	0.571
2DL5	1.1 (0.6–2.1)	0.793	3.2 (1.1–9.5)	0.039	1.3 (0.7–2.2)	0.420
2DS3	0.4 (0.1–1.3)	0.131	2.6 (0.9–7.5)	0.087	0.7 (0.3–1.6)	0.452
3DS1	1.0 (0.5–2.1)	0.892	3.3 (1.1–9.9)	0.032	1.2 (0.7–2.2)	0.449
2DS5	1.6 (0.8–3.2)	0.221	2.6 (0.8–7.9)	0.103	1.7 (0.9–3.1)	0.093
2DS1	1.1 (0.6–2.1)	0.793	3.2 (1.1–9.5)	0.039	1.3 (0.7–2.2)	0.420
Donor *KIR* haplotype						
A/A	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
B/x	1.3 (0.7–2.5)	0.447	4.2 (1.3–13.5)	0.016	1.5 (0.9–2.7)	0.134

Abbreviations: allo-HCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; Bu, busulfan; CD, cluster of

differentiation; CI, confidence interval; CMV, cytomegalovirus; CR, complete remission; HCT-CI, hematopoietic cell transplant-comorbidity index; HR, hazard ratio; KIR, killer-cell immunoglobulin-like receptor; Mel, Melphalan; NCR, non-complete remission; rATG, rabbit anti-thymocyte globulin

Table 4. Multivariable Analyses for Relapse, Non-relapse Mortality, and Overall Survival in CR and NCR Patients

	Relapse		Non-relapse Mortality		Overall Survival	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
(A) CR Patients						
Model 1						
KIR2DS1-positive donor (vs. *KIR2DS1*-negative donor)	0.1 (0.0–0.8)	0.030	0.5 (0.1–2.7)	0.426	0.2 (0.1–0.8)	0.022
Patient age (per 10 years)	0.8 (0.5–1.3)	0.338	1.3 (0.7–2.3)	0.366	1.0 (0.7–1.4)	0.973
Model 2						
KIR2DS1-positive donor (vs. *KIR2DS1*-negative donor)	0.1 (0.0–0.8)	0.027	0.7 (0.1–4.3)	0.659	0.2 (0.0–0.8)	0.026
Infused $CD34^+$ cell dose [$\times 10^6$/kg]						
Tertile 1	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
Tertile 2	0.4 (0.1–1.7)	0.209	3.3 (0.4–29.1)	0.283	0.8 (0.3–2.6)	0.731
Tertile 3	1.0 (0.2–5.7)	0.965	1.2 (0.1–21.7)	0.889	1.1 (0.2–5.1)	0.922
Model 3						
KIR2DS1-positive donor (vs. *KIR2DS1*-negative donor)	0.1 (0.0–0.9)	0.044	0.4 (0.1–2.1)	0.298	0.2 (0.1–0.8)	0.019
Female donor to male patient	0.8 (0.2–3.8)	0.768	1.2 (0.2–6.2)	0.794	0.9 (0.3–2.8)	0.851
Model 4						
KIR2DS1-positive donor (vs. *KIR2DS1*-negative donor)	0.1 (0.0–1.0)	0.052	0.7 (0.1–4.1)	0.668	0.3 (0.1–1.0)	0.047
HCT-CI ≥ 3 (vs. HCT-CI 1-2)	1.4 (0.3–6.6)	0.707	4.8 (0.9–25.2)	0.061	2.4 (0.8–7.3)	0.124

Model 5						
KIR2DS1-positive donor (vs. *KIR2DS1*-negative donor)	0.1 (0.0–1.2)	0.069	0.5 (0.1–2.5)	0.375	0.3 (0.1–0.9)	0.035
rATG (vs. No rATG)	3.1 (0.8–11.3)	0.089	2.4 (0.6–10.3)	0.230	2.7 (1.0–7.0)	0.045
(B) NCR Patients						
Model 1						
KIR2DS1-positive donor (vs. *KIR2DS1*-negative donor)	1.1 (0.6–2.3)	0.737	3.0 (0.9–9.4)	0.062	1.2 (0.7–2.2)	0.495
Patient age (per 10 years)	1.0 (0.8–1.2)	0.770	1.1 (0.7–1.7)	0.712	1.0 (0.8–1.3)	0.737
Model 2						
KIR2DS1-positive donor (vs. *KIR2DS1*-negative donor)	0.8 (0.4–1.7)	0.597	2.9 (0.8–10.3)	0.093	0.9 (0.5–1.8)	0.843
Infused $CD34^+$ cell dose [$\times 10^6$/kg]						
Tertile 1	1.0 (Ref)	–	1.0 (Ref)	–	1.0 (Ref)	–
Tertile 2	0.3 (0.1–0.8)	0.013	0.3 (0.1–1.7)	0.173	0.4 (0.2–0.8)	0.011
Tertile 3	0.3 (0.1–0.7)	0.008	0.8 (0.2–2.9)	0.691	0.5 (0.2–1.0)	0.054
Model 3						
KIR2DS1-positive donor (vs. *KIR2DS1*-negative donor)	1.0 (0.5–1.9)	0.917	3.1 (1.0–9.5)	0.047	1.2 (0.7–2.1)	0.616
Female donor to male patient	2.8 (1.4–5.8)	0.004	1.1 (0.3–4.3)	0.836	1.9 (1.0–3.5)	0.047
Model 4						
KIR2DS1-positive donor	1.1 (0.6–2.2)	0.734	2.8 (0.9–8.7)	0.067	1.2 (0.7–2.2)	0.490

(vs. *KIR2DS1*-negative donor)						
HCT-CI ≥ 3	0.8 (0.4–1.7)	0.565	1.7 (0.6–4.9)	0.322	1.2 (0.7–2.2)	0.488
(vs. HCT-CI 1-2)						
Model 5						
KIR2DS1-positive donor	1.1 (0.6–2.2)	0.757	3.2 (1.0–9.5)	0.041	1.3 (0.7–2.3)	0.403
(vs. *KIR2DS1*-negative donor)						
AML (vs non-AML)	2.1 (1.0–4.3)	0.039	1.2 (0.4–3.5)	0.691	1.9 (1.1–3.5)	0.032
Model 6						
KIR2DS1-positive donor	1.1 (0.6–2.1)	0.808	3.4 (1.1–10.4)	0.032	1.3 (0.7–2.2)	0.424
(vs. *KIR2DS1*-negative donor)						
rATG	1.1 (0.5–2.1)	0.846	0.7 (0.2–2.2)	0.549	1.0 (0.6–1.8)	0.994
(vs. No rATG)						

Abbreviations: AML, acute myeloid leukemia; CD, cluster of differentiation; CI, confidence interval; CR, complete remission; HCT-CI, hematopoietic cell transplant-comorbidity index; HR, hazard ratio; KIR, killer-cell immunoglobulin-like receptor; NCR, non-complete remission; rATG, rabbit anti-thymocyte globulin

Figure legends

Figure 1. Cumulative incidence of relapse (CIR), non-relapse mortality (NRM), acute graft-versus-host disease (GVHD), and Kaplan-Meier estimate of overall survival (OS) according to donor *KIR2DS1* positivity. (A) CIR in complete remission (CR). (B) CIR in non-complete remission (NCR). (C) Cumulative incidence of NRM in CR. (D) Cumulative incidence of NRM in NCR. (E) OS in CR. (F) OS in NCR. (G) Cumulative incidence of acute GVHD grade II or higher in CR. (H) Cumulative incidence of acute GVHD grade II or higher in NCR.

Figure 2. Cumulative incidence of relapse (CIR), non-relapse mortality (NRM), acute graft-versus-host disease (GVHD), and Kaplan-Meier estimate of overall survival (OS) according to tertiles of infused $CD34^+$ cell dose (Tertile 1, 2.1–3.7; Tertile 2, 3.8–5.6; and Tertile 3, 5.7–37.8 [$\times 10^6$/kg]). (A) CIR in complete remission (CR). (B) CIR in non-complete remission (NCR). (C) Cumulative incidence of NRM in CR. (D) Cumulative incidence of NRM in NCR. (E) OS in CR. (F) OS in NCR. (G) Cumulative incidence of acute GVHD grade II or higher in CR. (H) Cumulative incidence of acute GVHD grade II or higher in NCR.

Figure 1

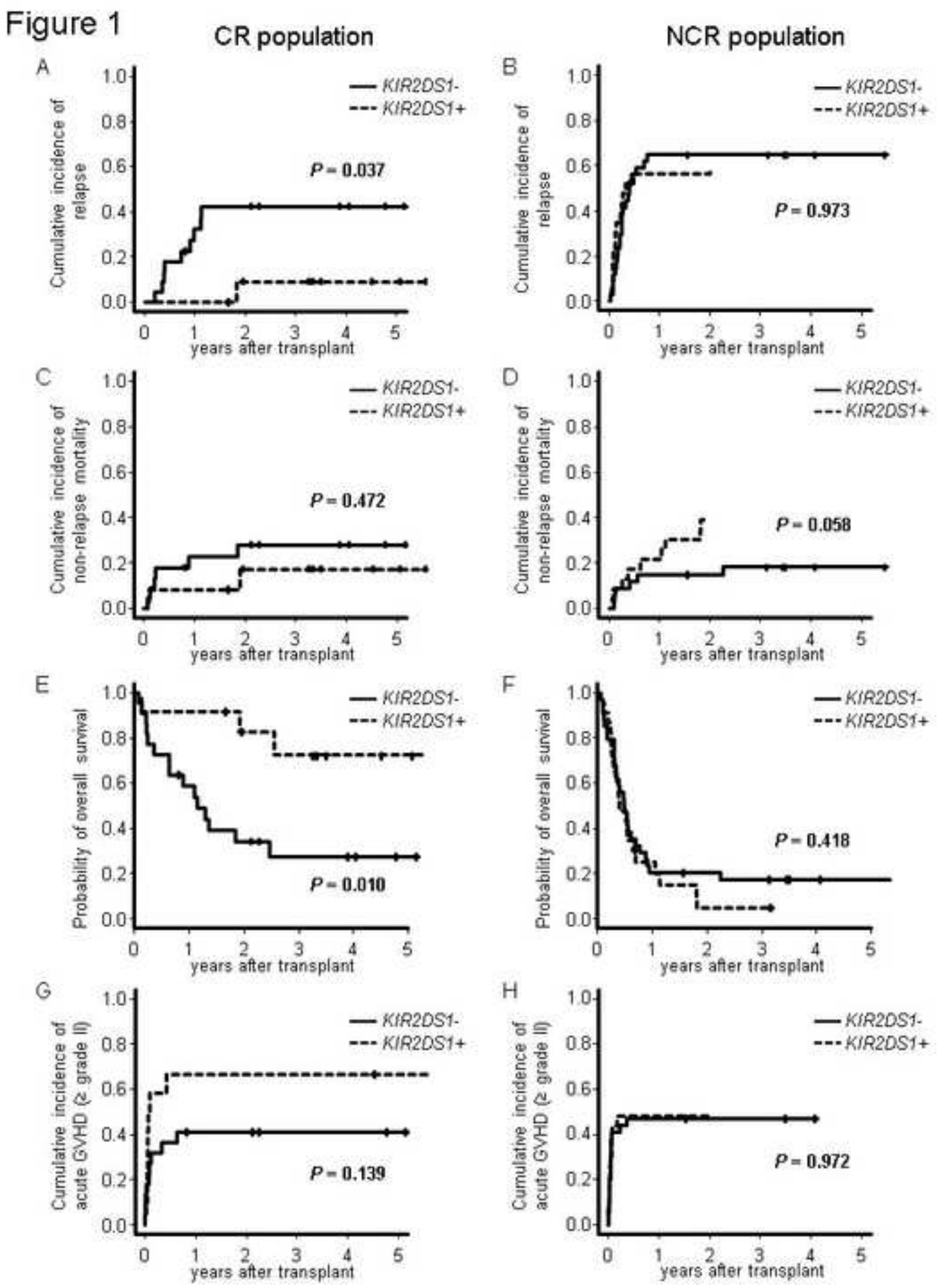
Figure 1
CR population
NCR population
A
B
C
D
E
F
G
H
KIR2DS1-
KIR2DS1+
Cumulative incidence of relapse
Cumulative incidence of non-relapse mortality
Probability of overall survival
Cumulative incidence of acute GVHD (≥ grade II)
years after transplant
P = 0.037
P = 0.973
P = 0.472
P = 0.058
P = 0.010
P = 0.418
P = 0.139
P = 0.972

Figure 2

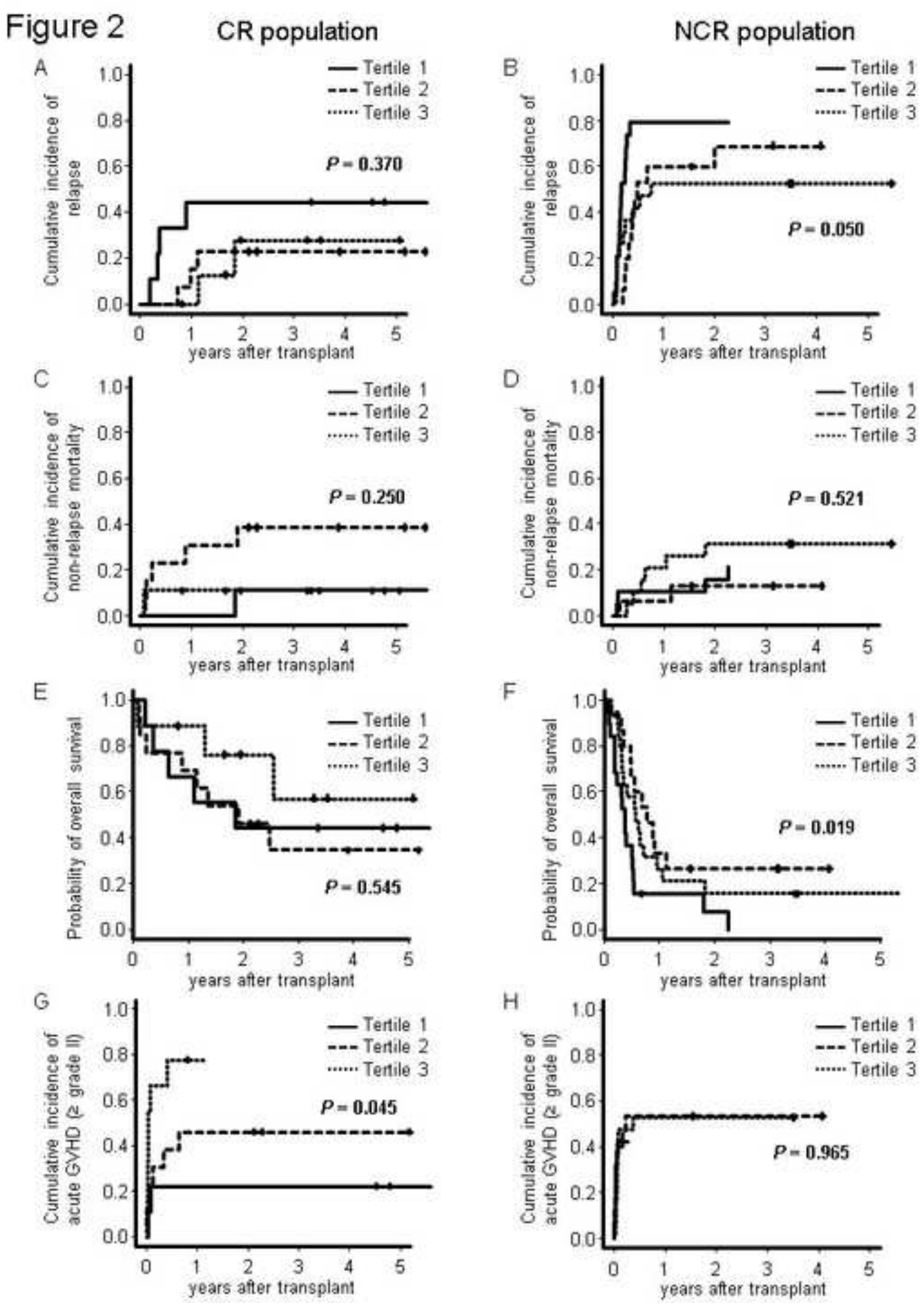
Figure 2
CR population
NCR population
A
B
C
D
E
F
G
H
Tertile 1
Tertile 2
Tertile 3
Cumulative incidence of relapse
Cumulative incidence of non-relapse mortality
Probability of overall survival
Cumulative incidence of acute GVHD (≥ grade II)
years after transplant
P = 0.370
P = 0.050
P = 0.250
P = 0.521
P = 0.545
P = 0.019
P = 0.045
P = 0.965

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