

The Effect of HLA-DRB1 allele Mismatch on the Results after Hematopoietic Stem Cell Transplantation

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Abstract :

Introduction.

We evaluated the effect of HLA-DRB1 allele mismatch on the results of HSCT in patients with haematological malignancies.

Patients and methods.

We compared 43 patients with an HLA-DRB1 allele-mismatched (DRB1-MM) donor (5/6 match) with 86 patients with an HLA-A, -B, and -DRB1 matched unrelated donor (6/6 MUD). The effect of an additional HLA-C mismatch was also studied. The two groups were well matched for all major characteristics such as year of HSCT, age, disease, disease risk index (DRI), stem cell dose, and conditioning.

Results.

The 5-year overall survival was 52% and 62% ($p=0.27$), and relapse-free survival was 42% and 47% ($p=0.18$) in DRB1-MM and MUD patients, respectively. Non-relapse mortality at one year was 12% and 15%, respectively ($p=0.55$). The cumulative incidence of relapse at 5 years was 46% in DRB1-MM patients and 35% in MUD patients ($p=0.03$). However, additional HLA-C mismatch affected the result negatively in the DR-mismatch cohort but not in the MUD cohort.

Conclusion.

The results suggest that an isolated HLA-DRB1 allele mismatch is acceptable but that additional mismatches may be detrimental.

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Introduction

The number of hematopoietic stem cell transplantations (HSCTs) with alternative donors has increased in recent years. However, polymorphism of the HLA genes is a major obstacle to HSCT, as HLA-A, -B, -C, and -DRB1 incompatibilities increase the risk of acute GVHD and mortality¹⁻³. Today, even though we are usually able to find a fully matched volunteer donor for most patients, a significant number of patients still lack such donor. The degree of high-resolution matching at HLA-A, -B, -C, and -DRB1 loci influences outcome after HSCT¹⁻³. Some previous studies have shown that HLA allele mismatch significantly affects the clinical outcome after unrelated donor HSCT^{4,5}. However, it has been proposed that in vivo T-cell depletion with anti-thymocyte globulin (ATG) may reduce the deleterious effects of HLA mismatch⁶⁻⁸.

In this study, we wanted to determine the effect of an HLA-DRB1 allele mismatch on outcome after HSCT in patients with HLA-A and -B matched unrelated donors with ATG incorporated in the conditioning. The effect of additional HLA-C mismatch was also studied.

Patients and methods

Study patients

We included all the patients with a haematological malignancy who were consecutively treated with hematopoietic stem cell transplantation (HSCT) at Karolinska University Hospital with an HLA-A and -B identical but HLA-DRB1 allele-mismatched unrelated donor between 1994 and 2015. Most patients had acute myeloid leukaemia (AML) (n=18) or acute lymphoid leukaemia (ALL) (n=10). Other diagnoses were myelodysplastic syndrome (MDS) in four patients, chronic myeloid leukaemia (CML) in 5, lymphoma in 5, and rhabdomyosarcoma in 1 (Table 1). For disease-risk index (DRI) classification, disease indication in combination with disease stage at time of HSCT (and cytogenetic data for AML and MDS patients) were used to

classify patients into the low (n=5, 12%), intermediate (IM, n=21, 49%) or high risk (n=17, 40%) patient cohorts according to Armand et al.⁹.

The study was approved by the Research Ethics Committee of Karolinska Institutet (DNR 425/97). The procedures were in accordance with the Helsinki Declaration.

Controls

As controls, we selected patients transplanted using an HLA-A, -B, and -DRB1 matched (at allele level) unrelated donor (MUD). The controls were matched for age, diagnosis, disease stage, year of HSCT, and conditioning. Two controls were selected for each study patient. By DRI, there were 11 low-risk patients (13%), 49 intermediate-risk patients (57%), and 26 high-risk patients (30%) in the control group.

HLA-typing

All patients and donors were typed using high-resolution molecular typing (i.e. PCR-SSP) for both HLA class I and II genes at allele level (at two fields level). All patient-donor pairs have recently been retrospectively re-typed.

Conditioning

Conventional myeloablative conditioning (MAC) was given to 82 patients and consisted of cyclophosphamide (Cy), 60 mg/kg for two days, in combination with busulphan (Bu), 4 mg/kg for 4 days (n=32), or total-body irradiation (TBI) at 4 × 3 Gy (n=49). Reduced-intensity conditioning (RIC) consisted of fludarabine, 30 mg/m²/day for 5–6 days, in combination with Bu, 4 mg/kg/day for two days (n=27), Cy at 120 mg/kg (n=3), TBI at 2 × 3 Gy and Cy at 30 mg/kg for two days (n=12), or treosulphan at 12 g/m² for 3 days (n=6).¹⁰.

Most patients (n=126) were treated with anti-T-cell serotherapy before HSCT, with the last dose being given on the day before infusion of the graft. Serotherapy consisted of Thymoglobulin (n=111) (Genzyme,

Table 1. Characteristics of patients and donors included in the study comparing HSCT with HLA-DRB1 allele-mismatched donors or HLA-A, -B and -DR matched (MUD) unrelated donors

	MUD	DRB1 allele MM	p-value
No.	86	43	
Year of HSCT	2008 (1994–2015)	2007 (1994–2015)	0.87
Age, years	33 (2–65)	33 (1–65)	0.79
Sex, Male/Female	54/32	26/17	0.85
Diagnosis:			
AML/ALL	33/23	18/10	0.81
CML	10	5	
Lymphoma	10	5	
MDS	8	4	
Other malignancy	2	1	
Disease stage (Early/Late)	28/58	14/29	1
MAC/RIC	55/31	27/16	1
BM/PBSCs	33/53	13/30	0.44
TNC dose, (× 10 ⁸ /kg)	7.6 (0.6–47.5)	7.6 (0.5–20.4)	0.56
CD34 dose, (× 10 ⁶ /kg)	6.9 (0.8–45.2)	5.7 (1.2–66.0)	0.23
Donor age, years	33 (19–56)	35 (20–55)	0.12
Female to male	8 (9%)	8 (19%)	0.16
ATG	85	41	0.54
Thymo/Campath/other	75/5/5	36/3/2	1
Dose Thymo (mg/kg)	5.9 (3.7–13.4)	6.1 (3.9–12.5)	0.03
Additional HLA-C MM	23	21	0.07
Folow-up, years	8.3 (0.6–21.1)	9.4 (1.3–18.8)	0.49

AML, acute myeloid leukaemia; ALL, acute lymphoid leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; Early stage, CR1/CP1; Late stage, beyond CR1/CP1; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; BM, bone marrow; PBSCs, peripheral blood stem cells; TNC, total nucleated cell; Female to male, female donor to male patient; ATG, anti-thymocyte globulin; Thymo, Thymoglobulin®.

Cambridge, MA, USA), 2 mg/kg/day for 2–5 days, Alemtuzumab (n=8) (Genzyme), 30 mg, ATG-F (n=3) (Frezenius, Gräfelfing, Germany) at 5 mg/kg/day for five days, or OKT-3 (n=4) at 5 mg/day for 5 days¹¹. The dose of Thymoglobulin has been gradually reduced during the years as a result of continuous development of treatment protocols¹¹. The choice of ATG brand depended on availability and any possible allergy against rabbit.

GVHD prophylaxis

Most patients (n=110) were given cyclosporine (CsA) and methotrexate (MTX) as prophylaxis against GVHD. During the first month, CsA levels in blood were kept at 150–200 ng/mL. In the absence of GVHD, the aim was to continue CsA at four to five months. Tacrolimus (Tac) and sirolimus were given to 15 patients, and CsA + prednisolon and Tac + MTX to one patient each. Two patients received a T-cell-depleted graft.

Diagnosis and treatment of GVHD

Both acute and chronic GVHD were diagnosed on the basis of clinical symptoms and/or biopsies from the skin, liver, gastrointestinal tract, or oral mucosa according to standard criteria^{12–14}. The patients were treated for grade-I acute GVHD with prednisolone, starting at 2 mg/kg/day with tapering after the initial response. In more severe cases, ATG, methylprednisolone, Infliximab, MTX, mesenchymal stromal cells (MSCs), and—more recently—placenta-derived decidual stromal cells were used^{15–18}.

Stem cell source

The source of graft was peripheral blood stem cells (PBSCs) in 83 cases and bone marrow (BM) in 46 cases. Stem cells were mobilized with subcutaneous G-CSF daily for 4–6 days before aphaeresis to obtain PBSCs¹⁹.

Supportive care

Supportive care has been described in detail previously^{20,21}.

Definitions

Relapse was defined as recurrent appearance of disease after complete remission, or disease progression after partial remission or stable disease. Cytomegalovirus (CMV) infection was defined as > 1,000 CMV DNA copies/mL of whole blood detected by PCR and it was pre-emptively treated using ganciclovir or foscarnet²². CMV disease was defined by the presence of symptoms combined with the detection of CMV in the affected organ. The diagnosis of post-transplantation lymphoproliferative disease (PTLD) was made according to the histological criteria reported for B-cell lymphoproliferative states following transplantation²³.

Statistics

Overall survival (OS) and relapse-free survival (RFS) were calculated using the Kaplan-Meier method, and compared with the log-rank test. The incidences of transplant-related mortality (TRM), relapse, and GVHD were obtained using an estimator of cumulative incidence curves with competing events.

Uni- and multivariate predictive analyses of OS and RFS were performed using the Cox proportional hazards model. Univariate and multivariate analyses for non-relapse mortality (NRM), relapse, and GVHD were performed using the proportional sub-distribution hazard regression model with competing risks developed by Fine and Gray.²⁴ Factors with a p-value of < 0.10 in the univariate analysis were included in the backwards-elimination multivariate analysis. Factors analyzed were patient and donor age, sex mismatch, diagnosis, disease stage, DRI, graft cell dose, GVHD prophylaxis, HLA match, conditioning, and graft source.

Analyses were performed using the cmprsk package (developed by Gray, June 2001), Splus 6.2 (Insightful,

Seattle, WA, USA), and Statistica 12 software (StatSoft, Tulsa, OK, USA).

Results

In the study group, an additional HLA-C mismatch occurred in 21 cases (6/8 match). Thus, in 22 cases an isolated HLA-DRB1 mismatch was present (7/8 match).

In the MUD control group, an HLA-C mismatch occurred in 23 cases. In 57 cases, there was an 8/8 match. In six donors, HLA-C were not known. Patients with an HLA-DRB1 allele mismatch received a significantly higher ATG dose (median 6.1 mg/kg as opposed to 5.9 mg/kg, $p=0.030$).

Survival and non-relapse mortality

Overall survival at 5 years was 62% (95% CI 50

(RH=0.73, 95% CI 0.42–1.28, $p=0.55$), respectively. No significant effect of HLA-C mismatch on NRM was seen in the two groups (Figure 1B, Table 2). Causes of death in the MUD group were relapse in 18 cases (21%), infection in 12 cases (14%), and GVHD in three cases (3%). In the DRB1-MM cohort, 15 patients (35%) died of relapse, 4 (9%) died of infection, and one died of GVHD.

Donor age (HR=1.04, 95% CI: 1.01–1.08, $p=0.02$) was the only factor significantly associated with mortality in multivariate analysis, while having an HLA-DRB1 and HLA-C mismatched donor was borderline-significant (HR=1.88, 95% CI: 0.89–3.94, $p=0.08$) (Table 3). A donor age of > 45 years showed the worst OS ($n=18$, 28%), and there was a correlation between younger donor age and better OS (donor age 26–45 years: 63%; donor age 19–25 years: 77%).

Table 3. Results from multivariate analysis for different outcome variates after HSCT. Hazard ratio (HR), 95% confidence interval (CI), and p-value are given

Factor	Mortality (OS)	Relapse	Relapse or Mortality (RFS)
DRI, high	-	2.10, 1.09–3.70, $p=0.025$	1.71, 1.02–2.88, $p=0.044$
TNC dose	-	1.05, 1.01–1.08, $p=0.008$	1.04, 1.01–1.07, $p=0.004$
Donor age (cont.)	1.04, 1.01–1.08, $p=0.02$	-	-
MUD(8/8)	Reference	Reference	Reference
MUD + C-MM	0.89, 0.40–1.98, $p=0.78$	1.47, 0.61–3.52, $p=0.39$	1.28, 0.62–2.66, $p=0.50$
DRB1-MM	0.63, 0.25–1.58, $p=0.32$	1.04, 0.45–2.43, $p=0.92$	0.89, 0.43–1.85, $p=0.76$
DRB1 + C-MM	1.88, 0.89–3.94, $p=0.08$	2.92, 1.36–6.26, $p=0.006$	2.32, 1.21–4.45, $p=0.012$

Cont., continuous variable; MM, mismatch; OS, overall survival; RFS, relapse-free survival; DRI, disease risk index; TNC, total nucleated cell dose; MUD, HLA-A, -B, -C, and -DRB1 matched unrelated donor

–72%) in MUD patients and 52% (36–66%) in DRB1-mismatched (DRB1-MM) patients (HR=1.37, 95% CI 0.78–2.38, $p=0.27$). In the DRB1-MM cohort, an HLA-C mismatch affected survival, which was 71% (47–86%) in C-matched patients and 33% (14–52%) in C-mismatched patients, (HR=3.61, 95% CI 1.38–9.43, $p<0.01$) (Figure 1A). No effect of HLA-C mismatch was seen in the MUD cohort (Table 2). NRM was 15% (7–23%) and 12% (4–23%) at one year in the two groups

Relapse and relapse-free survival

The cumulative incidence of relapse at 5 years was 35% (25–46%) in the MUD patients and 46% (30–61%) in the HLA-DRB1-MM patients (1.75, 1.03–3.13, $p=0.030$). In the DRB1-MM cohort, relapse was 33% (14–54%) or 60% (34–79%) depending on whether an HLA-C match or an additional HLA-C mismatch was present (HR=2.21, 95% CI 0.93–5.24, $p=0.075$) (Figure 1C).

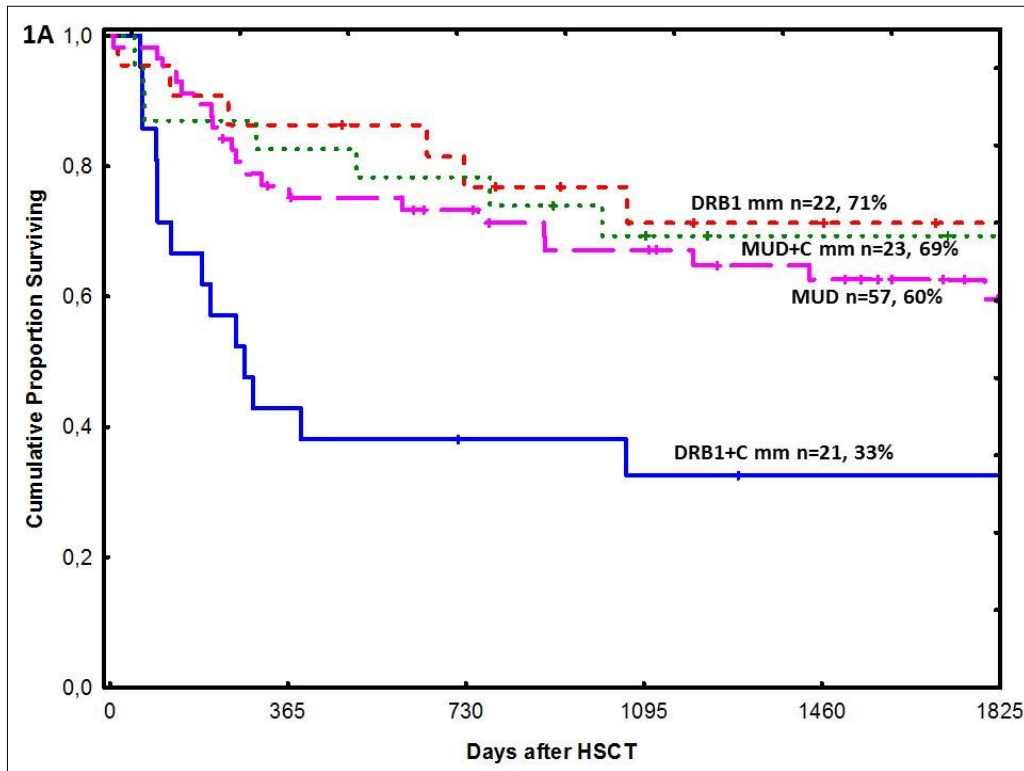


Figure 1A. Survival

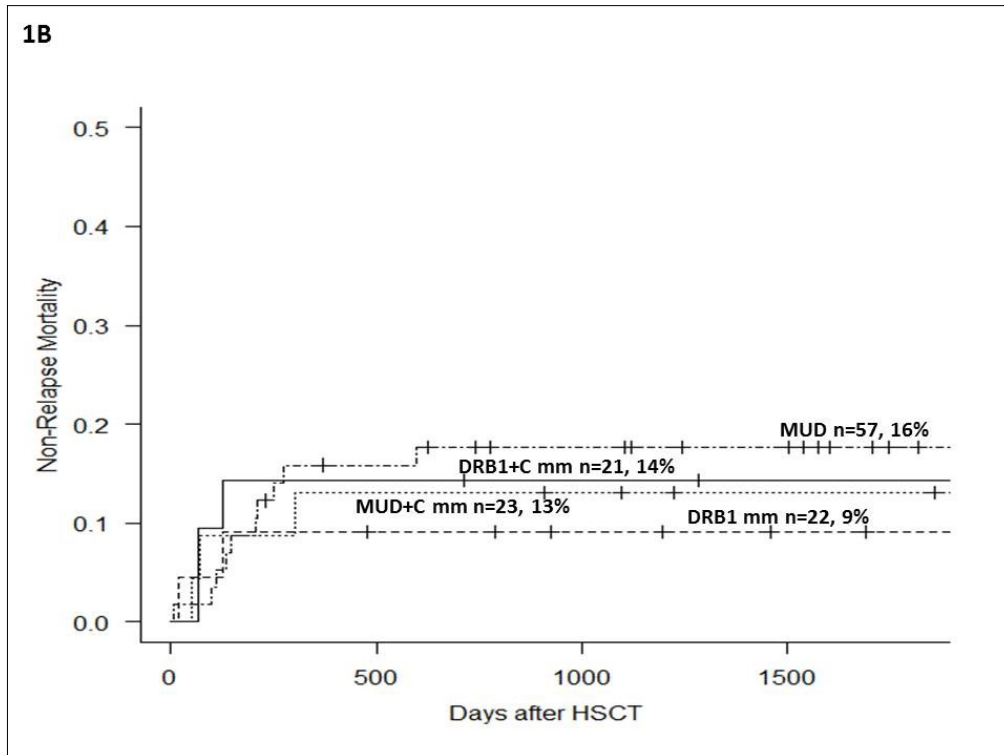


Figure 1B: non-relapse mortality

Table 2. The effect of an HLA-C mismatch in HSCT with an HLA-DRB1 allele-mismatched or HLA-A, -B, and -DR matched (MUD) unrelated donor

DRB1 allele MM	Add. MM	N=	OS	RFS	NRM	Relapse
	No	22	71% **	58% *	9%	33% #
	+C	21	33%	25%	14%	60%
MUD	No	57	60%	49%	16%	33%
	+C	23	69%	43%	13%	44%

Add. MM, additional HLA-C mismatch; MM, mismatch; OS, overall survival; RFS, relapse-free survival; NRM, non-relapse mortality.

* $p < 0.05$.

** $p < 0.01$.

$p = 0.07$ compared to additional HLA-C mismatch.

In multivariate analysis, there were correlations between relapse and an HLA-DRB1 and -C mismatched donor (HR=2.92, 95% CI 1.36–6.26, $p=0.006$), DRI high (HR=2.10, 95% CI: 1.09–3.70, $p=0.025$), and higher total nucleated cell (TNC) dose (HR=1.05, 95% CI: 1.01–1.08, $p=0.008$).

RFS at 5 years was 47% (36–58%) and 42% (27–57%) in the two groups, respectively (HR= 0.71, 95% CI 0.44–1.17, $p=0.18$). In the DRB1-MM cohort, HLA-C mismatch affected RFS: 58% (34–76%) in HLA-C-matched patients and 25% (9–44%) in HLA-C-mismatched patients (HR=2.44, 95% CI 1.08–5.56, $p=0.032$) (Figure 1D). No effect of HLA-C mismatch was seen in the MUD cohort (Table 2).

In multivariate analysis, there were correlations between lower RFS and having an HLA-DRB1 and -C mismatched donor (HR=2.32, 95% CI: 1.21–4.45, $p=0.012$), DRI high (HR=1.71, 95% CI 1.02–2.88, $p=0.041$), and high TNC dose (HR=1.04, 95% CI: 1.01–1.07, $p=0.004$).

Graft-versus-host disease and graft failure

The cumulative incidences of acute GVHD of grades II–IV were 41% (31–52%) and 28% (16–42%)

in the MUD and DRB1-MM groups, respectively (HR=0.60, 95% CI 0.32–1.14, $p=0.12$), and those of grades III–IV were 6% (2–12%) and 5% (1–14%) (HR=0.64, 95% CI 0.13–3.22, $p=0.57$). No significant effect of HLA-C mismatch was found (Figure 2A).

In multivariate analysis, higher donor age was associated with a lower incidence of moderate-to-severe acute GVHD (HR=0.66, 95% CI 0.44–0.96, $p=0.03$).

The incidences of chronic GVHD were 50% (37–61%) and 34% (19–50%) in the MUD and DRB1-MM groups, respectively (HR=0.73, 95% CI 0.38–1.37, $p=0.27$). No significant effect of HLA-C mismatch was found in the DRB1-MM group, while an HLA-C mismatch increased the incidence of chronic GVHD in the MUD group (38% vs. 77%; HR=2.65, 95% CI 1.38–5.07, $p=0.005$) (Figure 2B). In multivariate analysis, a parous female donor to male recipient (HR=3.10, 95% CI 1.28–7.52, $p=0.012$), higher patient age (HR=1.02, 95% CI: 1.01–1.03, $p=0.030$), and lower donor age (HR=0.96, 95% CI 0.93–0.99, $p=0.042$) were associated with chronic GVHD.

Graft failure/rejection occurred in two patients in the MUD group and in three patients in the DRB1-MM group.

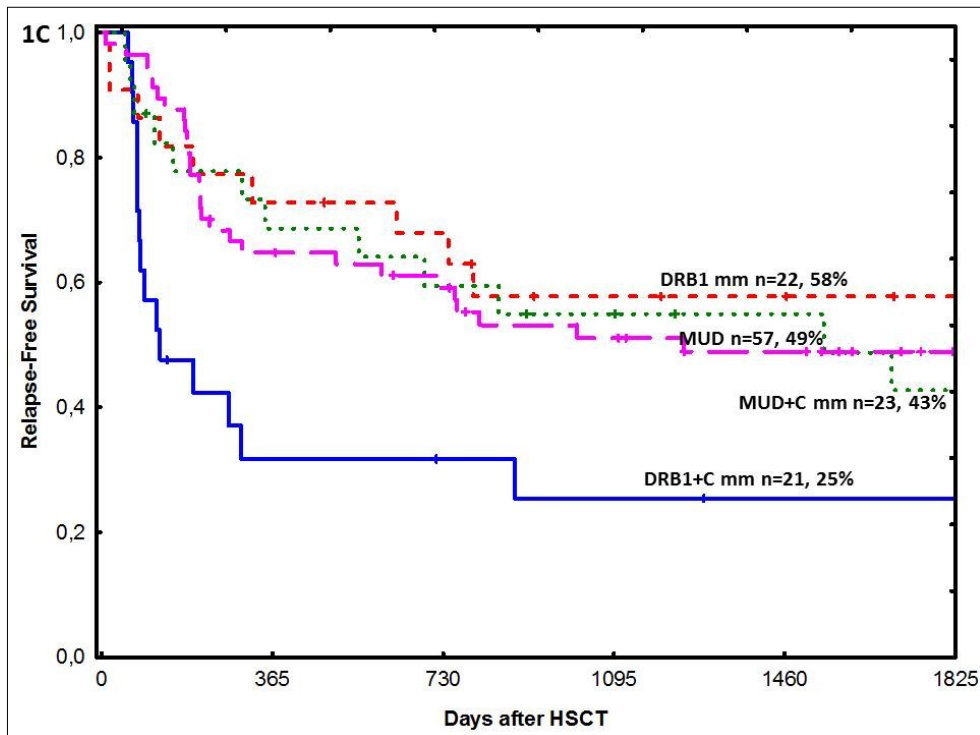


Figure 1C: relapse and relapse-free survival

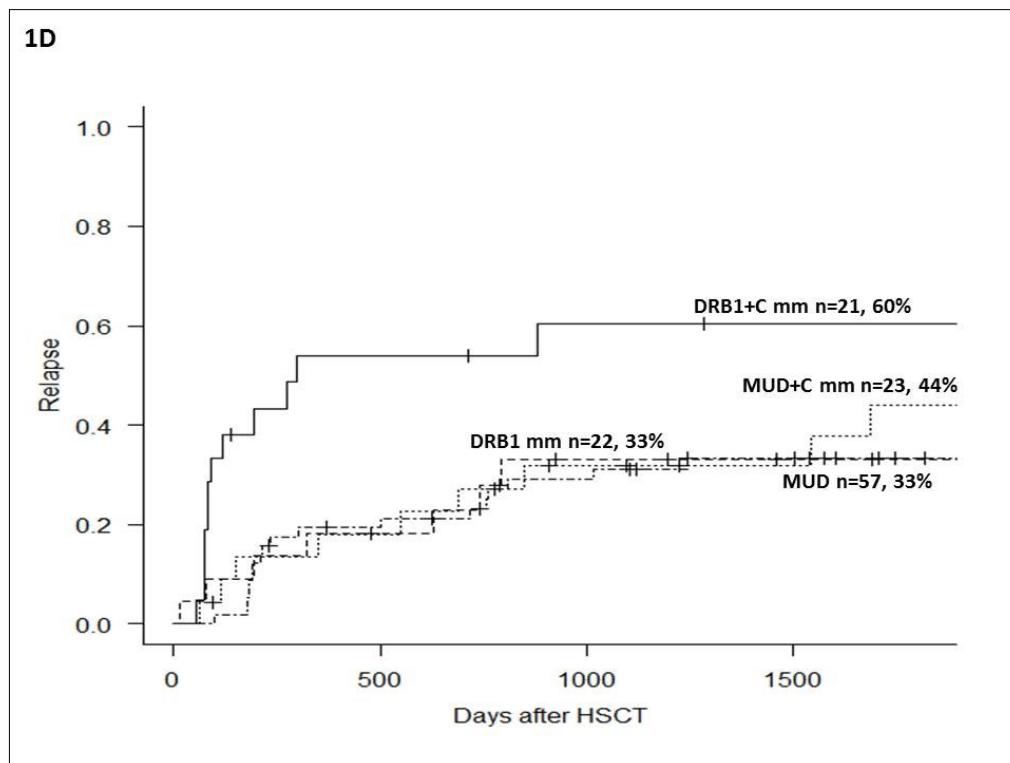


Figure 1D: after allogeneic stem cell transplantation in patients with unrelated donors, according to the type of HLA mismatch.

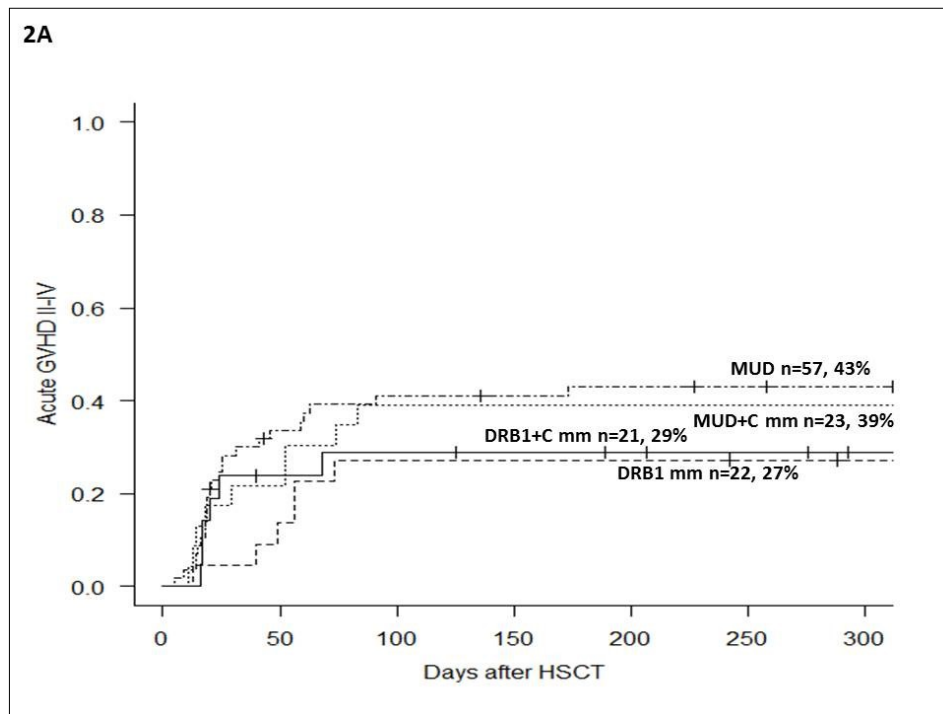


Figure 2A: Acute GVHD of grades II-IV (A) and chronic GVHD

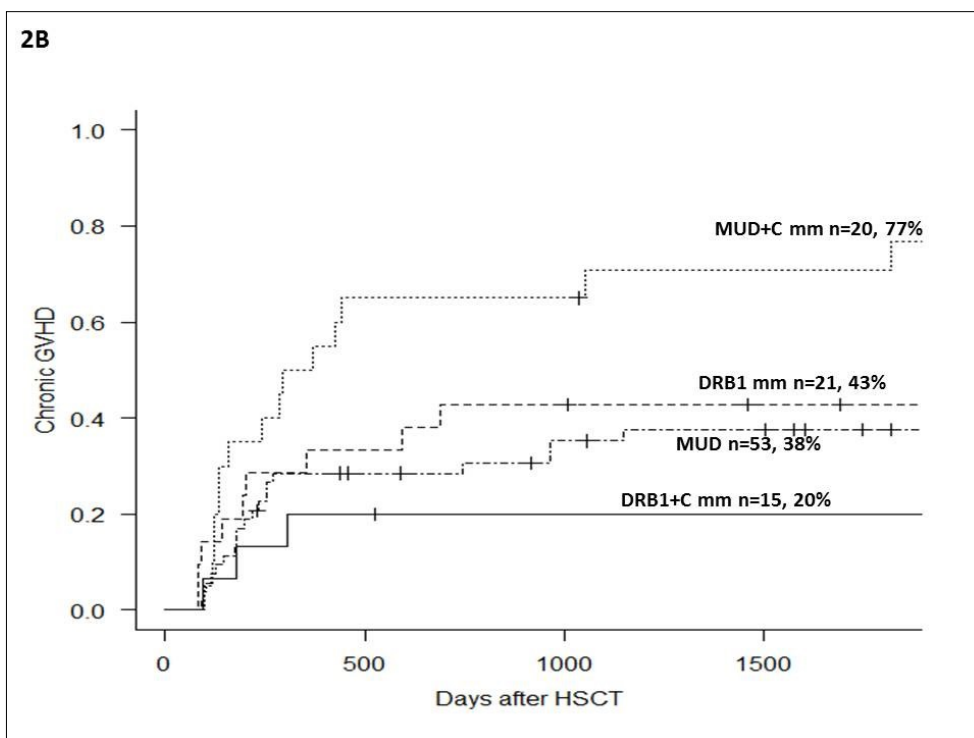


Figure 2B: chronic GVHD after allogeneic stem cell transplantation in patients with unrelated donors, according to the type of HLA mismatch.

Infections

No significant difference in infections was seen in the two groups. CMV reactivation occurred in 50% of the patients in both groups. CMV disease occurred in six patients (7%) in the MUD group and in four patients (9%) in the DRB1-MM group. Epstein-Barr virus-related post-transplantation lymphoproliferative disease was seen in 5% of the patients in both groups.

No effect of ATG dose and brand was seen on any of the results in this study.

Discussion

In this study, we evaluated the effect of an HLA-DRB1 allele mismatch on the results after unrelated donor (URD) HSCT and compared these results to those in a matched cohort of patients with an HLA-A, -B, and -DRB1 allele-matched URD. The study was retrospective, rather small, and included patients transplanted during a rather long period of time and the results should therefore be interpreted with caution.

We did not include HLA-DQ typing, as these results were available from only half of the patients included in the study. For this reason, we cannot exclude the possibility that HLA-DQ mismatch may affect the results in unrelated donor HSCT.

Some studies have evaluated the effect of any HLA-A, -B, -C, or -DRB1 allele mismatch, with different results. Kröger et al. found no effect of HLA-A, -B, -C, -DRB1, or -DQB1 allele mismatch in URD HSCT²⁵. Parody et al. found that an HLA-A, -B, -C, or -DRB1 allele mismatch resulted in more acute GVHD, but with no difference in OS, RFS, and NRM²⁶. Fuji et al. found more acute GVHD, inferior OS, and higher NRM if an HLA-A, -B, or -DRB1 allele mismatch occurred after related-donor HSCT²⁷. A Japanese study evaluated the effect of antigen and allele mismatch after URD HSCT and found lower RFS and higher NRM in mismatched unrelated donors²⁸. An Italian study found better OS, lower NRM, and less acute GVHD in 10 out of 10 matched URD

HSCTs, as compared to 8–9 out of 10 matched URD HSCTs²⁹.

Two studies found that a single HLA-DRB1 allele mismatch did not affect the outcome after URD HSCT^{2,30}. However, other studies have found a negative effect of a single HLA-DRB1 allele mismatch on the outcome after HSCT^{1,31,32}.

In the present study there was no significant difference in OS, RFS, NRM, and GVHD between the HLA-DR MM cohort and the controls.

Interestingly, the study group (HLA-DRB1 mismatch) had a higher incidence of relapse but a trend to lower incidence of GVHD compared to the controls. This balanced out as similar OS and RFS. On the face of it, this might seem counter-intuitive, but the outcome of any HSCT is dependent not only on donor factors but how the transplant is carried out.

The increased incidence of relapse in the HLA-DRB1 mismatched patients may be an effect of the weak trend of lower incidence of GVHD. It is well known that GVHD has a graft-versus-leukaemia effect, resulting in less relapse in patients with acute and chronic GVHD^{33,34}. Another reason could be that we might have immune-suppressed these patients too much with a higher dose of ATG and perhaps longer CsA treatment due to the mismatch³⁵. However, inclusion of ATG dose and time on CsA in the multivariate analysis did not have any effect on the results.

In multivariate analysis, an HLA-DRB1 and -C mismatch, DRI high and a higher total nucleated cell (TNC) dose were correlated to relapse and relapse-free survival (Table 3).

An additional HLA-C mismatch was detrimental in patients with an HLA-DRB1 allele-mismatched donor, but not in the MUD patients (Table 2). A negative effect of a single HLA-C mismatch and also an increased effect of multiple mismatches have been reported previously. Six large multicenter studies involving > 1,800 patients

have found an increased mortality risk with an HLA-C mismatch, and an effect of multiple mismatches^{1,2,30-32,36}. A stronger effect of an HLA-C antigen mismatch than of an allele mismatch was reported in most of these studies. We were not able to evaluate this effect, as only eight of our HLA-C mismatches were at the allele level. In patients with an isolated HLA-DRB1 mismatch (a 7/8 match), excellent results were seen — but there were few patients in this group (n=12) (Table 2).

Having an older donor was associated with less acute GVHD II–IV in the present study. An older donor may have a less active immune system through a different phenotype and function of CD8 T-cells — resulting in less GVHD but more infections and more relapse, leading to lower survival³⁷.

Our results may indicate that a single HLA-DRB1 mismatched donor leads to results comparable to when an 8/8-matched URD is used. However, an additional HLA-C mismatch may be detrimental.

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