Hematopoietic Stem Cell Transplantation: Contrasting the outcome of transplantations from HLA-identical siblings, partially HLA-mismatched related and HLA-matched unrelated donors*.

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*Dedicated to Prof. Dr. med. Ulrich W. Schaefer, Director of the Department of Bone Marrow Transplantation, University Hospital of Essen, who died on the 18th of August, 2002.

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Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) is a proven curative therapy for many hematological malignancies. HSCT from HLA-identical sibling donors (ISD) is still the golden standard. For the remaining 70% of the patients lacking an ISD alternative (partially) HLA-matched family donors (MFD) and HLA-matched unrelated donors (MUD) are now widely accepted. However, it is presently unclear whether outcome after HSCT from MFD or MUD is superior. Thus, the classical clinical endpoints after HSCT from ISD (n=138), MFD (n=86), and MUD (n=101) were compared by means of uni- and multivariate statistical analyses. MFD transplants with HLA class II (DRB1±DQB1) mismatches in graft-versus-host (GvH) direction showed an increased risk of grades II-IV graft-versus-host disease, and MFD transplants with more than a single HLA class I (A±B±C) mismatch in host-versus-graft (HvG) direction were associated with a higher risk of graft failure. However, no significant difference in overall survival was detectable between the three study groups after adjustment for the main predictors of transplant outcome. Thus, for patients lacking an ISD, an already identified MFD with an HLA-DRB1±DQB1 mismatch in GvH- or a combined HLA-A±B±C mismatch in HvG-direction should only be accepted in clinical urgent settings leaving no time to identify a MUD.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established curative therapy for a variety of hematological malignancies [1]. Genotypically HLA-identical sibling donors (ISD) - being available for about 30 % of the Caucasian patients [2] – are still regarded as the best donors for HSCT [3]. However, for the remaining 70 % of patients alternative donors, i.e. (partially) HLA-matched family donors other than HLA-identical siblings (MFD) and HLA-A,B,C,DRB1,DQB1 matched unrelated (MUD) donors are meanwhile routinely accepted [4 - 7].

Fact is that the clinical outcome after HSCT from MFD [4, 8, 9] as well as MUD [10 - 13] has meanwhile clearly improved, probably due to progress made in the domains of HLA-typing techniques [14 - 16] and supportive therapy [17, 18].

However, it is presently unclear, whether HSCT from MFD or from MUD has a superior outcome since the above cited clinical studies do not directly compare these two allogeneic approaches. Thus, the question is still open how to proceed if the donor search among the patients' siblings - which is always run first - has only identified a MFD, but no ISD. Should any or at least a subgroup of MFD (which remains to be defined) be accepted immediately without further effort to identify a MUD? Or should an unrelated donor search, even if expensive and time consuming, be run for all these patients?

The present clinical study directly compares the classical clinical endpoints of transplant outcome from MFD and MUD with ISD transplants serving as controls and identifies two subgroups of MFD which should not be accepted routinely for HSCT.
Patients, Donors, and Methods

**Entry criteria.** Enrolled were all patients transplanted at our institution during the period form January 1, 1994 to July 31, 2000 if fulfilling the following entry criteria: (1) patient age $\geq 16$ years, (2) allo- transplantation for chronic myeloid (CML), acute myeloid (AML), or acute lymphatic (ALL) leukemia, (3) first transplant, i.e. no preceding allo- or auto- transplant, (4) no graft manipulation (e.g., no T cell depletion), (5) myeloablative conditioning regimen used (containing 4 x 2.5 Gy fractionated total body irradiation and cyclophosphamide), (6) cyclosporine plus short course methotrexate protocol used for GvHD prophylaxis, and (7) transplantations performed under strict regimens for gut decontamination of anaerobic bacteria and reverse isolation (laminar air flow conditions). At the time of analysis all enrolled patients ($n = 325$) who survived had a follow up of $\geq 18$ months.

**Strategy of donor search and selection of graft source.** The strategy of donor search was in accordance with the “First German Consensus on Immunogenetic Donor Search” [19]. Thus, for patients lacking an ISD, an MFD was accepted (without a further effort to identify a MUD) if matched with the patient in GvH direction for at least five out of the six HLA- A, B, DRB1 antigens (irrespective of the number of additional HLA-mismatches in HvG-direction). If more than one MFD was available we tried to avoid selection of a female donor for a male patient and of a CMV negative donor for a CMV positive recipient. Since October 1994, we preferred peripheral blood over marrow as graft source in patients with an increased risk of graft failure and/or with a recent serious infectious disease as outlined previously [9].
Histocompatibility studies. HLA class I (A, B, C) typing of patients and donors relied on conventional serology (supplemented by 1D-IEF) until 1996 and on low resolution DNA-based typing (PCR-SSP) thereafter. For the purposes of the present study > 90% of donors and recipients belonging to the MFD group were retyped for HLA-A, B, C by low resolution PCR-SSP. For identification and selection of HLA class II (DRB1, DQB1) matched donors, the MLC test was routinely used until 1994, supplemented by HLA-DR, DQ serology. After 1994, the DRB1, DQB1 antigens of all donors and recipients were identified according to the German consensus on immunogenetic donor search [19]. Thus, for patients and donors belonging to the ISD and MFD group low resolution PCR-SSP (generic level, two digit code) was used, supplemented by a high resolution typing technique in case of ambiguous results (e.g., homozygosity). In the MFD setting German experts did not feel a need for routine high resolution HLA-DRB1, DQB1 typing, if low resolution typing has already disclosed a donor/recipient DRB1 and/or DQB1 mismatch (e.g. DRB1 010x vs. 040x) and the test results were supported by a pedigree plot segregation analysis. In contrast high resolution PCR-SSP (allelic level, four digit code) was routinely employed in the unrelated donor-recipient setting.

Baseline characteristics of study transplants. The following variables were used for initial (i.e., at presentation) characterization: type of donor (MUD yes = 1 vs. other = 0, and in parallel MFD yes = 1 vs. other = 0), patient sex, donor sex, sex mismatch (female donor for male recipient = 1, other = 0), patient age, donor age, ABO blood group major incompatibility, underlying disease (CML, AML, ALL), disease stage (early = CML first chronic phase and AML/ALL first complete remission = 0 vs. advanced = all other stages = 1), and graft source (peripheral blood = PB = 0 vs. bone marrow = BM = 1). For all
MFD transplants the following initial parameters were documented additionally: Donor/recipient HLA match/mismatch as detailed by HLA locus (A, B, C, DRB1, and DQB1) and involved immunologic vector (GvH, HvG direction), sum of HLA class I as well as sum of HLA class II mismatches in GvH- and in HvG-direction.

**Outcome characteristics of study transplants.** In order to document transplant outcome the following variables were selected: overall survival (OS), treatment related mortality (TRM), engraftment, grades II-IV acute graft-versus-host disease (aGvHD), and relapse (REL). For all outcome variables time post transplant to event was documented together with the corresponding status variable (event/censored). Engraftment was assumed if self-sustaining blood neutrophil counts greater than 1,000/µL together with untransfused platelet counts > 20.000/µL were reached by day 28 post transplant. The grades of aGvHD were assessed according to the published standard criteria [20]. The diagnosis of relapse was established by cytomorphology and/or cytogenetics.

**Univariate statistical analysis.** For direct comparison of initial parameters of the three study groups the Kruskal-Wallis test was used for skewed continuous variables (such as time to therapy), a one-way ANOVA for symmetrically distributed continuous variables (such as age), and the Chi-square test for independence for categorical variables. To illustrate the dependence of time to event of the three transplant groups (ISD, MFD, MUD) Kaplan-Meier diagrams were used. If “stage of disease” was the dominating influence parameter the corresponding Kaplan-Meier diagram is shown after stratification for disease stage. Since all Kaplan-Meier diagrams are shown for illustrative rather than inferential analysis, p-value have been deferred to the multivariate analysis.
**Multivariate statistical analysis.** The influence of all baseline variables listed in Table 1 on the times to achieve the analytical endpoints of transplant outcome was evaluated by Cox proportional hazard regression with backward elimination of parameters with a p-value above 0.2. For the analysis of interactions, the variables of main interest “MFD: yes/no” and “MUD: yes/no” were forced to remain in the Cox model. Interactions between the variables “MFD: yes/no” and “MUD: yes/no” and the identified risk factors in the final Cox model were evaluated by including interaction terms (e.g., “patient age * MFD” and “patient age * MUD”) one at a time and testing whether the explained deviation of the model exceeded the 0.95-quantile of the chi-square-distribution with one degree of freedom.

For graft failure as an event, the scarcity of events did not allow a higher-dimensional analysis without severe risk of biased estimation. Therefore, an exploratory analysis of the biologically plausible variables was performed in order to test whether the obtained results are within the range of results reported by previously studies.
Results

1. Overall Survival (OS)

**Univariate statistical analysis** showed the stage of disease and the age of the patient to have a marked influence on OS after HSCT. The 5-year OS rate, for example, reached 67% for patients with early disease, but declined to 29% for patients with advanced disease. The corresponding data for OS were 65% for patients aged ≤ 37 years) but dropped down to 47% for patients older than 37 years. In contrast, univariate analysis could not demonstrate any impact on OS of all other initial characteristics listed in Table 1, including the type of donor used. In order to illustrate the results of univariate analysis, the OS curves for the three study group transplants (ISD, MFD, and MUD) are shown in Figure 1 after stratification for the dominating variable “stage of disease”.

**Multivariate statistical analysis** revealed the parameters “disease stage”, “patient age”, “time interval between diagnosis and transplantation” as well as “donor age” to be independent risk factors for OS (cf. Table 3). In contrast, the type of donor was excluded from the Cox model for OS, irrespective of whether tested as “MUD: yes/no” (p = 0.6) or as “MFD: yes/no” (p = 0.4). For comparison with the explanatory parameters, listed in Table 3, the hazard ratios [95% confidence intervals] for the excluded parameters “MUD” and “MFD” were 1.17 [0.7 - 1.7] and 1.15 [0.7 - 1.7], respectively.

Thus, in contrast to advanced disease and advanced patient age, the type of donor (ISD, MFD or MUD) had no significant impact on OS after HSCT in our study.
2. Treatment-related/Non relapse-related mortality (TRM)

**Univariate statistical analysis.** TRM was found to be clearly influenced by disease stage and patient age. The 5-year TRM, for example, was 38 % for patients with early but 60 % for patients with advanced disease, and 25 % for patients aged ≤ 37 years as compared to 48 % for patients older than 37 years. In contrast, the other initial variables listed in Table 1 including the type of donor had no significant influence on TRM. To illustrate the latter finding, we calculated the TRM for ISD, MFD and MUD group patients after stratification for the dominating risk factor, i.e. disease stage. For patients with early disease we documented a 5-year TRM of 27 %, 29 % and once again 29 % in the ISD, MFD, and MUD group, respectively. The corresponding percentages for patients with advanced disease were 55 %, 58 %, and 65 %, respectively.

**Multivariate statistical analysis.** Cox model building suggested three of the initial transplant characteristics listed in Table 1 to be independent risk factors for TRM, namely “disease stage”, “patient age” and “donor age” (cf. Table 3). In contrast, the type of donor was eliminated from the Cox model irrespective of whether tested as “MUD: yes/no” (p = 0.5) or “MFD: yes/no” (p = 0.2). The calculated hazard ratios [95 % confidence intervals] for the excluded parameters “MUD” and “MFD” were 1.2 [0.7 - 1.9] and 1.3 [0.8 - 2.0], respectively.
3. Graft Failure (GF)

Only patients surviving day 28 post transplant (n = 318) were included into the analysis. Notably, no case of GF was observed in the 136 patients transplanted from an ISD, whereas GF occurred in 8 of the 84 patients transplanted with an MFD and in 5 of the 98 patients grafted with a MUD.

The Kaplan-Meier procedure was used to evaluate the impact of the type of donor on the risk of primary GF on day 28 post transplant. The calculated risk of GF differed significantly between the three groups (ISD: 0 %, MFD: 8.5 % and MUD: 5.2 %; p < 0.004, log-rank test). In contrast, the difference between the MFD and MUD group was not significant.

To study the influence of the other base line variables listed in Table1 on GF, cross table calculations including all evaluable MFD and MUD group patients (n = 182) were performed. This type of analysis revealed the variable “graft source ( BM vs. PB )” to have an impact on the risk of GF. In the MUD group, BM was used in 74 and PB in 24 cases, and all observed 5 cases of GF were within the BM subgroup. In the MFD group, BM was used in 38 and PB in 46 cases. Of the 8 cases with graft failure, seven were within the BM and only one in the PB subgroup. For MFD patients the observed difference in the graft failure rate between BM and PB was significant (two sided Fisher`s exact test: p = 0.02).

Thus, the use of an MFD or a MUD instead of an ISD is clearly associated with a higher risk of GF.
4. Acute Graft-versus-Host Disease (aGvHD)

**Univariate statistical analysis** disclosed the risk of grades II-IV aGvHD to be influenced by three parameters, namely the type of donor (as depict in Figure 2), patient age (≤ 37 years: 37 %, > 37 years: 60 %), and donor age (≤ 38 years: 39 %, > 38 years: 57 %).

**Multivariate statistical analysis** confirmed the above results since it suggested (p < 0.2) the following initial variables as independent risk factors for grades II-IV aGvHD: type of donor “MFD”, type of donor “MUD”, “patient age”, “donor age”, and “disease stage” (cf. Table 3). The analysis of interactions between the parameters of main interest “MFD” and “MUD” and the other risk factors of the final Cox model revealed, that the introduction of the interaction variable “MFD * Patient age” improved the so far presented Cox model significantly (increase of Chi-square > 3.841).

Thus, the risk of aGvHD is clearly increased after HSCT from MFD as well as from MUD as compared to ISD and appears to be especially high in case of patients with advanced age transplanted from MFD.

5. Relapse (REL)

**Univariate statistical analysis** identified several variables to influence the risk of REL. The stage of disease had the greatest impact (early: 19 %, adv. 33 %), followed by the type of donor (ISD: 29 %, MFD 19 %, MUD 16 %), and the graft source (PB: 15 %, BM: 27 %). Figure 3 illustrates the calculated risk of relapse for ISD, MFD, and MUD group patients after stratification for the dominating influence variable “stage of disease”. Of
major interest was the high risk of REL in ISD group patients with early disease as compared to MFD and MUD group patients with early disease.

**Multivariate statistical analysis** confirmed the results given above since it suggested (with \( p < 0.2 \)) the following independent risk factors for relapse: “disease stage”, type of donor “MUD”, type of donor “MFD”, “graft source”, and “sex mismatch” (cf. Table 3). Thus, the use of a MFD or a MUD instead of an ISD seems to be protective (hazard ratio < 1) against relapse at least in patients with early disease.

6. Impact of HLA-mismatches

The data presented above demonstrated MFD and MUD group patients to be at a higher risk of aGvHD and of graft failure as compared to ISD group patients. However, the MFD group was very heterogeneous with regard to the pattern of donor/recipient HLA mismatches, as detailed in Table 1. Thus, we decided to evaluate whether the documented increased risk of aGvHD and of GF of the MFD group can be attributed to transplants with special patterns of donor/recipient HLA mismatches.

**Impact of HLA-mismatches on aGvHD**

The Kaplan-Meier procedure was used to calculate the risk of aGvHD for the following subgroups of transplants: “MFD class I” = MFD transplants with one or more HLA class I mismatches but no HLA class II mismatch in GvH-direction (\( n = 41 \)), “MFD class II” = MFD transplants with no class I but one or two class II mismatches in GvH direction (\( n = 21 \)), “MFD no MM” = MFD transplants with no class I and no class II mismatch in GvH-direction (\( n = 16 \)), and MUD transplants. Outcome data of ISD transplants served as
controls. As shown in Figure 4 the aGvHD risk was found to be highest in the “MFD class II” group (75 %), intermediate in the “MFD class I” (61 %) as well as MUD (46 %) groups, and lowest in the ISD (40 %) and the “MFD no MM” (28 %) groups.

Multivariate statistical analysis was confined to the enrolled MFD and MUD transplants (n =187). The type of donor was tested as “MUD: yes/no”, “sum of HLA-class I mismatches in GvH-direction (A+B+C)”, and “sum of HLA-class II mismatches in GvH-direction (DRB1+DQB1), respectively. Multivariate analysis suggested the “sum of HLA class II mismatches” (p < 0.001) and “patient age” (p = 0.08) as independent risk factors for aGvHD after HSCT from MFD or MUD with hazard ratios [95 % confidence interval] of 2.1 [ 1.5 - 3.1 ] and 1.02 [ 0.99 - 1.04 ], respectively. In contrast, the variables “MUD” and “Sum of HLA class I mismatches” were eliminated from the Cox model with p values of 0.5 and 0.6 and hazard ratios of 1.2 [ 0.6 - 2.5] and 1.1 [ 0.7 - 1.7 ], respectively.

Thus, transplantations from MFD mismatched for HLA-class II (DRB1±DQB1) antigens in GvH-direction imply a higher risk of grades II-IV aGvHD as compared to transplants from MFD mismatched for HLA-class I (A,B,C) antigens in GvH-direction or from MUD. Due to the high linkage disequilibrium between HLA-DRB1 and -DQB1 isolated DRB1 and DQB1 donor/recipient disparities were to rare to allow for an analysis of the impact of single locus HLA class II mismatches on aGvHD.
Impact of HLA-mismatches on primary GF

All MFD (n = 83) and MUD (n = 98) group patients surviving day 28 post transplant were included into the analysis. Firstly, cross table calculations for the occurrence of GF were performed in patients with 0, 1, 2 and 3 HLA class I (A+ B+C) mismatches in HvG direction, irrespective of the presence or absence of additional HLA class II mismatches in HvG direction. Consequently, all evaluable MUD group patients figured with 0 mismatches. The rate of GF was shown to increase significantly with the number of HLA class I disparities from 6/125 (4.8 %) and 1/28 (3.6 %) for patients with nil and one mismatch to 3/22 (13.6 %) and 3/6 (50%) for patients exhibiting two and three mismatches, respectively (p = 0.003, two sided Fisher`s exact test). In contrast, HLA class II disparities had no influence on primary GF after adjustment for class I disparities class II disparities.

Finally, we tested the impact of the sum of HLA class I (A+B+C) and the sum of HLA class II (DRB1+DQB1) mismatches in HvG direction on GF by Cox model building. The sum of HLA class II mismatches was not found to have an impact on GF (p = 0.7, hazard ratio 1.1 [0.5-2.3], whereas the variable „sum of HLA class I mismatches in HvG-direction” appeared as a prominent risk factor for GF with a p value of < 0.001 and a hazard ratio of 2.6 [1.6-4.2]. Thus, MFD transplants with more than one HLA class I (A ± B ± C) mismatch in host-versus-graft (HvG) direction are associated with a higher risk of graft failure as compared to other subgroups of MFD transplants or to transplants from MUD.
Discussion

The present retrospective single centre study compares three different approaches of HSCT as practiced at our institution, namely HSCT from genotypically HLA-identical siblings (ISD), related donors other than ISD (MFD) and HLA-matched unrelated volunteers (MUD). The main aim of the study was to answer the question of whether HSCT from MFD or from defined subgroups of MFD have an inferior clinical outcome as compared to MUD.

Most differences in initial characteristics between the three study cohorts documented in Table 1 were an integral part of the evaluated procedures, e.g., the higher mean donor age in the MFD group (since the patients’ parents served as MFD in multiple cases) or the lower frequency of sex mismatched transplants in the MUD group (since exclusion of sex mismatched donors was only feasible when several donors were available for one patient, which is a rare event in the ISD and MFD settings). Nevertheless, all differences in initial characteristics were regarded as possibly confounding variables. Thus, results suggesting an impact of the parameter “type of donor used” on clinical outcome were only accepted if confirmed by multivariate analysis.

The sample size of the presented single center study appears to be sufficient with regard to the study objective, especially because the scientific question has not been adequately considered by other studies. However, even if the quality of the presented data allows for making valid statistical predictions, we are aware that the number of cases enrolled is comparatively low in view of the clinical impact of our main conclusions. This applies especially to our finding that overall survival appears to be similar between the three study groups (cf. Figure 1 and Table 3).
Our finding of a similar long-term overall survival between the three study groups is surprising at least at first glance since at the same time the present study demonstrated an increased risk of primary GF (cf. results, section 3) as well as acute GvHD grades II-IV (cf. results, section 4) in the MFD and MUD as compared to the ISD group, and GF as well as clinically relevant aGvHD are well known live threatening complications after HSCT.

However, our findings are in accordance with those of a recent large multicenter study, comparing the outcome after ISD and MUD transplants for CML [21] and may be explained as follows. In a statistical analysis focusing on long-term OS after HSCT the impact of GF on OS may become “invisible” for the following reasons: (1) Primary GF is a rare event even in the MFD (8.5 %) and MUD (5.2 %) groups and occurs early after transplant. Thus, more frequent causes of death may “dilute” the impact of GF on OS at the long term, and (2) patients being successfully re-transplanted after GF are notably not censured in the Kaplan-Meier analysis for OS. Furthermore, the adverse effect of acute GvHD on OS after transplantation from MFD or MUD seem to be compensated at the long-term by the documented reduced risk of relapse at least in patients transplanted for early disease (cf. Figure 3). Anyhow, our finding of a similar OS after transplantation from ISD, MFD and MUD allows for two conclusions, (1) MFD and MUD donors, as defined by this study, are both acceptable in principal, if an ISD is not available and (2) the world-wide efforts in building up “unrelated marrow donor registries” were worthwhile [6, 22-24].

Another important result of this study is that two subgroups of MFD transplants had an inferior outcome as compared to the ISD and MUD groups: (1) Donor/recipient HLA-
class II (DRB1±DQB1) antigen mismatches in GvH-direction were associated with an increased risk of clinically relevant aGvHD (cf. Figure 4) and (2) HLA-class I (A±B±C) mismatches in HvG-direction (especially if combined) resulted in an elevated risk of graft failure. Thus, for patients lacking an HLA-identical sibling donor, an available related donor mismatched for HLA-DRB1±DQB1 in GvH- or for HLA-A±B±C in HvG-direction should only be selected in clinical urgent settings leaving no time to identify a possibly available MUD.

Additionally, our study gave a detailed insight into the biology of HLA in the context of allogeneic HSCT from related donors. Obviously, HLA class II mismatches in GvH-direction play a key role for the development of aGvHD, whereas HLA class I mismatches in HvG direction have a major impact on the risk of graft failure. Of note, our findings on HLA biology in the related setting are identical to those of a recent study from Seattle analysing a large cohort of unrelated transplants [25]. Another point of interest is the relatively high relapse rate for the early disease group (cf. Figure 3). A straightforward explanation for this phenomenon is that the use of an MFD or a MUD is only protective against relapse in case of early disease, but not in case of (generally more aggressive) advanced disease stages.

Finally, the data of this study indirectly contribute to the recent discussion of whether donor/recipient HLA-A,B,C sequence based typing (SBT) [26 - 28] will improve the outcome after unrelated HSCT without compromising donor availability [29, 30]. Our present data clearly argue against the routine use of SBT. We did not employ this time-consuming typing technique and could nevertheless achieve the same long term OS results.
after transplantation from MUD and “golden standard” ISD. Of interest is that a recent editorial published in the New England Journal of Medicine [31] endorses our present view. Nevertheless, our data on the value of SBT are still preliminary, since our outcome results were reached under special supportive therapy modalities [32] (strict reverse isolation and consequent gut decontamination of anaerobic bacteria).

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References


Legends to Figures

**Figure 1. Overall survival** (Kaplan-Meier estimates) after allogeneic hematopoietic stem cell transplantation from genotypically HLA-identical siblings (ISD), alternative (partially) HLA matched family donors (MFD), and HLA-matched unrelated volunteers (MUD). Results are given after stratification for early (ear) and advanced (adv) disease stage.

**Figure 2. Risk of acute graft-versus-host disease** (Kaplan-Meier estimates) after hematopoietic stem cell transplantation from genotypically HLA-identical siblings (ISD), alternative (partially) HLA matched family donors (MFD), and HLA-matched unrelated volunteers (MUD).

**Figure 3. Risk of relapse** (Kaplan-Meier estimates) after allogeneic hematopoietic stem cell transplantation from genotypically HLA-identical siblings (ISD), alternative (partially) HLA matched family donors (MFD), and HLA-matched unrelated volunteers (MUD). Results are given after stratification for early (ear) and advanced (adv) disease stage.

**Figure 4. Impact of mismatched HLA-loci on the risk of acute graft-versus-host disease** (Kaplan Meier estimates). Class I MM: 1 A or 1 B or 1 C or 1 B + 1C mismatch, but no class II mismatch in GvH-direction. Class II MM: 1 DRB1 and/or 1 DQB1 mismatch, but no class I mismatch in GvH-direction. For comparison, the outcomes after transplantation from HLA-identical siblings (ISD) and HLA-matched unrelated donors (MUD) are also shown.
Overall Survival

Fig. 1

Years post Transplant

MFD ear (n = 48)
MUD ear (n = 66)
ISD ear (n = 110)
ISD adv (n = 28)
MUD adv (n = 35)
MFD adv (n = 38)
Fig. 2

Acute GvHD Grades II - IV

Days post Transplant

- ▲ MFD (n = 86)
- ▼ MUD (n = 101)
- □ ISD (n = 138)
Fig. 3

Relapse

Years post Transplant

p

- ISD adv (n = 28)
- MUD adv (n = 35)
- ISD ear (n = 110)
- MFD adv (n = 38)
- MFD ear (n = 48)
- MUD ear (n = 66)
Fig. 4

Mismatched HLA loci and aGvHD II - IV

- MFD-MM II (n = 21)
- MFD-MM I (n = 41)
- MUD (n = 101)
- ISD (n = 138)
- MFD-no MM (n = 16)

Days post Transplant
Table 1. Initial Characteristics of Transplant Study Groups

<table>
<thead>
<tr>
<th></th>
<th>ISD</th>
<th>MFD</th>
<th>MUD</th>
<th>p*</th>
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<tr>
<td>N (total)</td>
<td>138</td>
<td>86</td>
<td>101</td>
<td></td>
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<tr>
<td><strong>Patient Age</strong></td>
<td></td>
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<tr>
<td>Median [Range] in years</td>
<td>40.5 [16-59]</td>
<td>35.5 [16-57]</td>
<td>40.0 [16-57]</td>
<td>*0.008</td>
</tr>
<tr>
<td>Age &gt; 37 years</td>
<td>84 (61%)</td>
<td>32 (37%)</td>
<td>58 (57%)</td>
<td>**0.002</td>
</tr>
<tr>
<td><strong>Patient Sex [m : f]</strong></td>
<td>79 : 59 (1.3 : 1)</td>
<td>49 : 37 (1.3 : 1)</td>
<td>58 : 43 (1.3 : 1)</td>
<td><strong>n.s.</strong></td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
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<td></td>
<td><strong>n.s.</strong></td>
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<td>107 (77 %)</td>
<td>56 (65 %)</td>
<td>76 (75 %)</td>
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<tr>
<td>AML</td>
<td>27 (20 %)</td>
<td>25 (29 %)</td>
<td>17 (17 %)</td>
<td></td>
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<tr>
<td>ALL</td>
<td>4 ( 3 %)</td>
<td>5 ( 6 %)</td>
<td>8 ( 8 %)</td>
<td></td>
</tr>
<tr>
<td><strong>Disease Stage</strong></td>
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<td></td>
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<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Early vs advanced</td>
<td>110 vs. 28</td>
<td>48 vs. 38</td>
<td>66 vs.35</td>
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<td>(80 % vs. 20 %)</td>
<td>(56 % vs. 44 %)</td>
<td>(65 % vs. 35 %)</td>
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<tr>
<td><strong>Interval: Diagnosis -&gt; TX</strong></td>
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<td>Median, Range [days]</td>
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<tr>
<td>CML</td>
<td>490 [38-5.162]</td>
<td>573 [111-4.057]</td>
<td>678 [102-5.182]</td>
<td>***&lt; 0.0001</td>
</tr>
<tr>
<td>AML/ALL</td>
<td>190 [72-1.293]</td>
<td>280 [120-1.589]</td>
<td>601 [104-2.003]</td>
<td>***0.001</td>
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<tr>
<td><strong>Stem Cell Source</strong></td>
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<tr>
<td>BM vs. PB</td>
<td>80 vs. 58</td>
<td>40 vs. 46</td>
<td>76 vs. 25</td>
<td>**&lt; 0.0001</td>
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<tr>
<td>(58 % vs. 42 %)</td>
<td>(46 % vs. 54 %)</td>
<td>(75 % vs. 25 %)</td>
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<tr>
<td><strong>Donor Age [years]</strong></td>
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<tr>
<td>Age &gt; 38</td>
<td>78/138 (56 %)</td>
<td>50/86 (58 %)</td>
<td>35/101 (35 %)</td>
<td>**0.001</td>
</tr>
<tr>
<td><strong>Donor Sex [m : f]</strong></td>
<td>63:75 (0.8 : 1)</td>
<td>38 : 48 (0.8 : 1)</td>
<td>65 : 36 (1.8 : 1)</td>
<td>**0.006</td>
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<tr>
<td><strong>Sex-Mismatch</strong></td>
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<td><strong>0.001</strong></td>
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<tr>
<td>Yes (donor f, patient m)</td>
<td>43/138 (31 %)</td>
<td>27/86 (31 %)</td>
<td>13/101 (13 %)</td>
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</tbody>
</table>

TX = Transplant, BM = Bone Marrow, PB = Peripheral Blood, f = female, m = male. * One way ANOVA, ** Chi-Square test, *** Kruskal-Wallis test. The p-values refer to differences between the three study groups with regard to the indicated baseline characteristics of transplants.
Table 2. HLA characteristics of transplants from HLA-matched family donors other than genotypically HLA-identical siblings (MFD study group)

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<td>DQB1</td>
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</table>

HLA matches (white boxes) and HLA mismatches (black boxes) are detailed by the HLA loci (A, B, C, DRB1 and DQB1) and the immunological vector (graft versus host = GvH, host versus graft = HvG). N = number of transplants for a given HLA constellation. More than 95% of the given HLA-mismatches were full antigen mismatches [e.g. A*02 vs. A*03, DRB1*01 vs. DRB1*04], whereas the remaining mismatches were HLA antigen split mismatches [e.g. B*15 (62) vs. B*15 (63), B*40 (60) vs. B*40 (61)].
<table>
<thead>
<tr>
<th>Clinical Endpoint</th>
<th>Baseline Variable</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>Overall Survival</strong></td>
<td>Disease Stage</td>
<td>3.31</td>
<td>2.3-4.6</td>
<td>&lt; 0.0001</td>
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<tr>
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<td>Patient Age</td>
<td>*1.03</td>
<td>1.01-1.05</td>
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<td>Interval Dg--&gt;TX</td>
<td>*1.09</td>
<td>1.00-1.2</td>
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<tr>
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<td>Donor Age</td>
<td>*1.01</td>
<td>0.99-1.02</td>
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<td><strong>Treatment-related Mortality</strong></td>
<td>Disease Stage</td>
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<td>2.3-5.0</td>
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<tr>
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<td>*1.04</td>
<td>1.02-1.06</td>
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<td>*1.02</td>
<td>1.00-1.03</td>
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<tr>
<td><strong>Acute Graft-vs-Host Disease</strong></td>
<td>Donor MFD</td>
<td>2.18</td>
<td>1.3-3.5</td>
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<td>1.72</td>
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<td>Disease Stage</td>
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<td><strong>Relapse</strong></td>
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<tr>
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<td>Graft Source</td>
<td>1.91</td>
<td>1.0-3.5</td>
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<tr>
<td></td>
<td>Sex Mismatch</td>
<td>0.42</td>
<td>0.2-0.9</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Patient age: patient age at transplant [years]. Donor age: donor age at transplant [years]. Interval Dg --> TX: time interval between diagnosis and transplant [years]. Disease stage: early = 0, advanced = 1. Sex mismatch: female donor for male patient = 1 versus other constellation = 0. Graft source: bone marrow = 1, peripheral blood = 0. Donor MFD: use of a matched family donor = 1 versus other type of donor = 0. Donor MUD: use of a matched unrelated donor = 1 versus other type of donor = 0.

* Note that the given hazard ratios are calculated on a “per year” basis.
Hematopoietic stem cell transplantation: contrasting the outcome of transplantations from HLA-identical siblings, partially HLA-mismatched related and HLA-matched unrelated donors

Hellmut D Ottinger, Stanislav Ferencik, Dietrich W Beelen, Monika Lindemann, Rudolf Peceny, Ahmet H Elmaagacli, Johannes Huesing and Hans Grosse-Wilde