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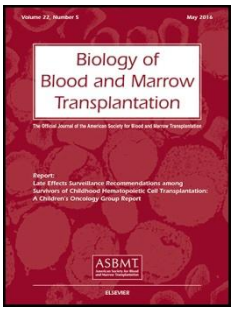

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Impact of HLA disparity in haploidentical bone marrow transplantation followed by high dose cyclophosphamide.

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Key points:

- Recipients of haploidentical transplantation may have a lower degree of HLA disparity, both global or in one-way allo-immune response
- Post-transplant cyclophosphamide leveled off HLA disparity, since a higher degree of HLA mismatches did not have any impact on outcome

Running Title: HLA disparity in haploidentical transplants

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HIGHLIGHTS

- Recipients of haploidentical transplantation may have a lower degree of HLA disparity, both global or in one-way allo-immune response
- Higher degree of HLA mismatches did not have any impact on OS, NRM, aGVHD, cGVHD ,relapse, graft failure.
- Advanced disease was the most significant predictor of poor outcome.

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Abstract

We studied the impact of HLA mismatching on the outcome of 318 consecutive patients who received an un-manipulated haploidentical bone marrow transplant, followed by post-transplant cyclophosphamide (PT-CY). The number of HLA mismatched antigens was tested for its impact on overall survival (OS) and non relapse mortality (NRM), whereas HLA mismatches in the graft versus host (GvH) direction were tested for prediction of GvHD and relapse; finally, we studied whether graft rejection correlated with the number of HLA mismatched antigens in host *versus* graft (HvG) direction.

Two-hundred and thirty-one donor/recipient pairs (72%) had 4/8 mismatches at the A, B, C, DRB1 HLA loci. HLA mismatches did not predict the 2 years OS(HR 0.83, p=0.58) and NRM(SHR 1.08, p=0.93). The cumulative incidence of aGvHD (p=0.13), 1-year cGvHD (p=0.84), and relapse rate (p=0.26) did not correlate with univectorial GvH mismatches.

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Similarly,no correlation was observed between the amount of HLA mismatch in the HvG direction and graft rejection.

In multivariate analysis, advanced disease at transplant was the strongest predictor of survival, NRM, relapse, and graft rejection.

In conclusion: the degree of HLA mismatching should not be used asa criterion to select family haplo-identical donors when using bone marrow as stem cell source and PT-Cy for GvHD prophylaxis.

Keywords: haploidentical transplantation; HLA disparity; post-transplant cyclophosphamide

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Introduction

Human leukocyte antigen (HLA) compatibility is the crucial requirement to perform an allogeneic hematopoietic stem cell transplantation (HSCT), and it remains the most meaningful predictor of long-term survival(1–3). Indeed, in patients undergoing HSCT, the number ofmismatched HLA is associated with higher rates of graft versus host disease (GvHD) and of non-relapse mortality (NRM)(3–5). Registry data have clearly demonstrated that even a single HLAmismatch may impact NRM, mostly due to increased GvHD or rejection(6,7). For this reason, in the presence of one or more HLA-mismatches (whether the donor is related or unrelated),GvHD prophylaxis is enhanced with pre-transplant anti-lymphocyte globulinor other lymphocyte-depleting agents(8–13).Nevertheless, the outcome of HSCT from mismatched donors remains less satisfactory, even if few studies have shown that the presence of HLA mismatchmay reduce relapse rate(14–16). These data discouraged the use of HSCT from donors with higher HLA-disparity (e.g., partially matched donors); indeed, HSCT from haploidentical related donors resulted in a high risk of rejection and of GvHD, with obvious impact on NRM, especially when HSC source is T-cell replete(2,17,18).

In recent years, different strategies have been adopted to improve the outcome of haploidentical HSCT, including protocols of graft manipulation *in vitro* (i.g. T-cell depleted HSCT)(19–22)and increased immunosuppression *in vivo* (i.e., T-cell repleted HSCT)(23–27). Among these, the use of post-transplant high dose cyclophosphamide (PTCy) allowed a large number of patients to receive a haploidentical T repleted HSCT, with acceptable GvHD and NRM and excellent long-term outcome(28–31).

HLA genes are inherited within parental haplotypes, which represent groups of physically linked alleles with possible conserved association due to positive "linkage disequilibrium"(32). By definition, "HLA-haplo-identical transplant'' means that familiardonor and recipient share only one inherited HLA-haplotype, while the second one is different and randomly derived.As a result, HLA disparity is supposed to be 50% (4 out of 8 HLA alleles, considering the

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HLA-A, -B, -C and –DRB1 loci) if the HLA alleles in the non-shared haplotypes are completely different. However, possible casual identity of some HLA alleles (due to a random heritage of homozygous allelesin the donor or in the recipient) may account for a lower degree of disparity. As a result, in some donor/recipient pairs the degree of disparity is less than the predicted 4 over 8 HLA alleles.

Furthermore, HLA incompatibility should be considered in a bi-directional fashion; the vector of incompatibility can certainly be seen from either the donor perspective (graft-versus-host, GvH direction) or from the recipient perspective (host-versus-graft, HvG direction). From the donor's perspective, the presence of recipient HLA-A, -B, -C, and -DRB1 differences not shared by the donor stimulates the donor anti-host allo-response or GvH recognition that is associated with higher risks of GvHD compared with complete matching. Conversely, from the patient's perspective, the presence of donor disparity not shared by the recipient provokes the HvGallo-response that increases the risk of graft failure. Thus, in the case of homozygosis of some HLA loci, reduced HLA-disparity may involve only one vector of the allo-immune response; this may affect GvHallo-response in the case of homozygosis of the recipient, or HvGallo-response in the case of homozygosis of the donor(32).

Even if it is now well established that PTCy allows a safe enough HSCT procedure across the HLA-barrier, we wondered whether a different HLA-matching may account for different outcome in the context of haploidentical bone marrow transplant (BMT). In this paper we report on 318 consecutive patients with high-risk disease and lacking an HLA identical donor, who received a BMT from a haplo-identical relatives in two Italian institutions after the same conditioning and GvHD prophylaxis regimen. This large series offers the opportunity to verify: 1) the effective degree of HLA mismatchbetween donor and recipient and 2) the possible impact of actual lower HLA disparity on HSCT outcome.

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Patients and Methods

We analyzed 318 consecutive patients with hematological malignancies transplanted in Genoa and Naples.All patients or their legal guardians signed approved informed consent to use their transplant data for clinical research as per EBMT standard before proceeding to transplantation. Patients were transplanted from August 2010 to July 2016 from a relatedhaploidentical donor, after receiving a myeloablative-conditioning regimen and GvHD prophylaxis as described below. Main eligibility criteria were: 1. Age 18-70 years; 2. Hematological malignancy with high risk of relapse (active disease at time of transplant was allowed); 3. Lack of a sibling HLA-identical donor; 4. Lack of an HLA-matched unrelated donor (at least 7/8) or suitablecord blood unit (at least 4/6, with ≥2 x 10^7 total nucleated cells [TNC]/kg) available in a clinically meaningful timeframe. Of these 318 patients, 294 were treated at San Martino Hospital in Genoa and 24 at Federico II University in Naples.

Donor selection

The best related haploidentical donor was selected according to the following algorithm: 1. Donor health; 2.Donor age; 3. CMV status (as compared to recipient's); 4. AB0-compatibility.The possibledifferent number of HLA-mismatches and relationship were not used as a criterion for donor selection.

Conditioning regimen

The myeloablative conditioning regimen was basedon either total body irradiation (TBI) or chemotherapy. TBI was given in three days (-8 to -6) at the total dose of 990 cGy or 1200 cGy(300 daily x 3, or 200 bi-daily x 3, respectively) and fludarabine 120 mg/m² in four days (-5 to -2) (n=56). The chemotherapy-only regimen consisted of: 1)Thiotepa 10 mg/kg in two days (-6-5), followed by fludarabine 150 mg/m² and Busulfan 9.6 mg/kg intravenously for three days (-4 to -2) (n=125). For patients aged more than 60 years, or patients with comorbidities or poor clinical condition, the dose of Busulfan was reduced to 6.4 mg/kg (one day was omitted)(n=120). 2)

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Thiotepa 10 mg/kg in two days, Fludarabine 50 mg/m² for three days, and Melphalan 70 mg/m2 (with myeloma or for patients previouslyautografted with Busulfan) (n=17).

GvHD prophylaxis

GvHD prophylaxis consisted of: 1. Post-transplant cyclophosphamide 50 mg/kg given on day +3 and +5; 2. cyclosporine A (CsA) 1 mg/kg given as a continuous iv infusion from days 0 to +20, adjusted for blood levels (200–400 ng/ml), and then orally until the day +180; 3. Mycophenolatemofetil (MMF) given orally at the dose of 15 mg/kg every 12 hours from day +1 to day +28. Mesna was administered according to institutional policies, at the minimum dose of 80% of the cyclophosphamide dose.

Stem cell source and transplantation

Un-manipulated bone marrow was used as stem cell support at day 0. Donors underwent bone marrow harvest under general anesthesia (Genoa) or epidural anesthesia (Naples) and the ideal target of total nucleated cells was 4×10^8/kg of recipient body weight. Pegylated-G-CSF 6 mg subcutaneouswasgiven on day +6 to all patients.

HLA typing

Donors and recipients were typed, until December 31st 2015, using DNA method (SSO and SBT) for HLA A, B, C, DRB1, DQ and DPB at a highresolution level, as defined by EFI standards.Starting from January 2016, HLA typing was doneby NGS at allelic level for the same loci. When applicable (72.3% of patients), other first- or higher-degree family members were typed to definitively establish haplotype identity and heritage.

Supportive care

Anti-infectious prophylaxis was started during the conditioning regimen and consisted of acyclovir 500 mg/m^2 three times a day, levofloxacin 500 mg a day, and fluconazole until day +75 (or mold-active prophylaxis for high-risk patients). Bi-weekly CMV monitoring, by PCR or antigenemia, was started on

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day −7, until day +100, and weekly until day +180. Patients received red blood cell and platelet transfusions according to institutional protocols.

Diagnosis and treatment of GvHD

The diagnosis of acute and chronic GvHD was mainly clinical, based on standard criteria(33,34), and confirmed, when possible, by histological analysis of skin and/or rectal biopsy specimens. First-line and second-line therapy for GvHD was provided according to institutional protocols.

Graft failure

Graft failure was defined as persistent pancytopenia with lack of donor chimerism (i.e., <5% donor) by day +30 from transplantation.

Assessment of HLA disparity

We assessed HLA incompatibility based on the total number of HLA mismatchesand their direction in each donor/recipient pair, and their correlation with the transplant outcomes. HLA mismatch in the GvH direction was defined as the presence of host antigens or alleles not shared by the donor. HLA mismatch in the HvG direction was defined as the presence of donor antigens or alleles not shared by the host. We evaluated overall survival (OS) and non-relapse mortality (NRM) according to the amount of overall mismatches; also, we analyzed cumulative incidence (CI) of grade II – IV acute GvHD (aGvHD), moderate-severe chronic GvHD (cGvHD), and cumulative incidence of relapse according to the degree of HLA mismatch in the GvH direction and graft rejection rate according to the degree of HLA mismatch in the HvG direction. For analysis purposes, the whole patient population was divided into 2 groups according to the number of HLA mismatch: 0-1-2 allele or antigen mismatches versus 3-4 allele or antigen mismatches (35). The same groups (0-2 versus 3-4 HLA mismatches) were also generated when analyzing vectorial HLA-disparity, either in the GvH or HvG direction. The analysis were performed grouping patients in 0-3 vs 4

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mismatches, bidirectional for OS and NRM, and monodirectional for GVHD and relapse (GvH vector) and graft failure (HvG vector).

Endpoints and statistical methods

Overall survival was estimated using the Kaplan-Meier approach; OS is defined as time from starting HSCT to death from any cause, or the last follow-up for living patients.Cumulative incidence analysis was used for NRM, relapse, and GvHD incidence (either acute or chronic). NRM is defined as death due to any cause other than progression of the underlying malignancy, with death due to relapse as competing event. Relapse is defined as recurrence of the underlying hematological malignancy; death due to any other cause (NRM) was a competing event for this analysis.

For GvHD cumulative incidence analysis, death without aGvHD in first 100 days was considered a competing event for aGvHD endpoint, while relapse or death in absence of cGvHD were considered as competing events for cGvHD outcome.

Association of mismatch and other demographic and clinical characteristics with overall survival was assessed using the univariable semiparametric Cox regression model, while the Fine and Gray model for competing risk was adopted to test the association with cumulative incidence of aGvHD, cGvHD, relapse, and NRM.

A univariable logistic regression model to determine association of demographic and clinical characteristics with rejection event was used.

The total number of mismatch was considered for OS and NRM, mismatch in GvHD direction was assessed for relapse, cGvHD, and aGvHD, while mismatch in HvG direction was considered for reject endpoint.

Hazard-ratios (HR) for the Cox model, Sub-hazard ratio (SHR) for the Fine and Gray model, and Odds-ratio (OR) for the logistic regression model were

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estimated and reported together with the corresponding 95% confidence interval (95% CI) to quantify the effect of single characteristics on outcomes.

For each outcome, a multivariable regression model was subsequently performed. All variables with a univariable p-value < 0.15 were considered for the stepwise selection, and those that werestatistically significant were included in the final, multivariable model.

A p-value ≤0.05 was considered statistically significant. Stata (v.13; StataCorp) was used for the computation.

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Results

Patients' characteristics

Complete baseline characteristics of transplanted patients are outlined in table 1 for all patients and according to the number of HLA mismatch. The main clinical features were balanced between the mismatch groups except for GvH direction group, which had more patients in an advanced phase of disease at transplant in the high mismatch group.

Median age of the 318 patients was 48 years (range 17-74). The patients were transplanted for the following underlying disease: acute myeloid leukemia (AML; n=130), acute lymphoblastic leukemia (ALL; n=64), lymphoproliferative disorders (LPD; n=43), chronic myeloproliferative neoplasm (CMPN; n=48), and myelodysplastic syndrome (MDS; n=33). About half of the patients (n=144, 45%) were in an advanced phase of disease at time of transplant.

HLA mismatches

Among the 318 donor/recipient pairs, 231 (73%) had 4/8 mismatches(A,B, C, and DRB1), but only 128(40%)had 4/8 "bidirectional" mismatches, while the remaining pairs shared one or more identical alleles at the examined HLA loci. Two- hundred and ninety-sixpatients(93%)presented a high number of HLA-mismatches (3 and 4; n= 65 and 231, respectively) with donor and 22 (7%) presented a low number (0-1-2). Table 2 reports in detail the number of total HLA-mismatch, as well as the degree of one-way incompatibility in GvH vector (3-4 HLA-mismatch=264, 83%; 0-1-2 HLA-mismatch=54, 17%) and HvG vector (3-4 HLA-mismatch=272, 85%; 0-1-2 HLA-mismatch= 46, 15%)of these 318 donor/recipient pairs.

The impact of HLA disparity (at HLA –A, -B, -C,-DRB1)was assessed in univariate analysis, taking into account the number of HLA mismatches (mm) as a discrete variable (0-2 vs 3-4 and 0-3 vs 4) and as a continuous variable (i.e.0,1,2,3,4 any direction;GvH vector and HvG vector, respectively).

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Transplant outcome

With a median follow up of 562 days (range 6-2241 days), 2-year OS was 58% (95% CI 52.5- 64%). Causes of deaths were relapse of disease for 74 (23%) patients and NRM for 61patients (18.8%) (infections: 38, hemorrhage: 7, GVHD: 12, endothelial complications:4).

Cumulative incidence of NRM was 16% (95% CI 12.1-20.2%) at 1 year.

Cumulative incidence of relapse was 29.8% (95% CI 24.7-35.1%)at 2 years.

Cumulative Incidence of aGvHD grade II-IV was 17.2% (95% CI:12.8%-22.1%)after 100 days from transplant with a rate of aGVHD grade III – IV of 5% (18 patients).

Cumulative incidence of moderate and severe cGvHD at 1 year was 13.9% (95% CI 10.1-18.4%). Graft failure occurred in 21 patients (6.6%).

OverallSurvival and Non Relapse Mortality

In univariate analysis,having more HLA differences was not associated with worse OS (fig. 1a) nor with increased NRM (fig.1b) (tab3), irrespective of whether they were analyzed as discrete (either 0-2 vs 3-4 mm and 0-3 vs 4 mm) or continuous variable. The variable associated with worse OS was an active disease at transplant (HR 3.61, $p<0.001$). Were predictive of NRM older age (SHR 1.03, $p=0.002$) and active disease at transplant (SHR 2.44, $p<0.001$). Multivariate analysis confirmed these data for OS and NRM (tab.4).

Acute GvHD and Chronic GVHD

In univariate analysis, having more HLA differences was not associated with a statistical difference in the risk of acute GvHD grade II – IV (Fig.2a) and chronic GVHD (fig.2b) (tab 5). The lack of association was observed when HLA mm were used as a discrete variable as well as when HLA mm was treated as a continuous variable, even if in this latter condition there was a trend for higher risk of aGvHD with increasing number of mismatches.

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None of the other transplant variables included in univariate analysis were predictive of acute or chronic GVHD.

Relapse

In univariate analysis, having more HLA differences was not associated with a statistical difference in the risk of relapse rate (Fig. 3) (Tab 5). The lack of association was observed when HLA mm were used as a discrete variable (both 0-2 vs 3-4 and 0-3 vs 4 mm), as well as when HLA mm was treated as a continuous variable. The variables associated with relapse were conditioning regimen (p=0.05) and active disease at transplant (HR 2.68, p<0.001). Also in multivariate analysis, conditioning regimen (p=0.035) and active phase of disease at transplant (HR 2.86, p<0.001) were associated with disease relapse (tab.4).

Graft failure

The number of patients who have less HLA mismatches with a donor was not protected from rejection. The variables associated with graft rejection were conditioning regimen (p=0.0078), active disease at transplant (HR 3.26, p=0.018), and older age (SHR 1.05, p=0.02) (tab 6). In multivariate analysis, graft rejection was associated with conditioning regimen (p=0.042)and with active disease at transplant (HR 2.79, p=0,05)(tab.4).

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Discussion

In this study, we have analyzed the actual degree of HLA mismatchesin the context of haploidentical HSCT, and its possible impact on transplant outcome. In our analysis, only 73% of donor/recipient pairs were mismatched for all 4 loci of the non-shared HLA haplotype. Thus, a significant proportion of transplants defined as haploidentical within the recent literature are more than half-matched, and may be better reported including their actual HLA disparity. However, this information does not clearly emerge from available data, raising possible concerns about the interpretation of haplo-HSCT results. Indeed, the impact of this possible reduced degree of disparity is as yet unknown. Based on well-established knowledge of transplant biology, these patients are expected to have a better outcome, since in the setting of unrelated transplants there is an obvious dose-effect of the number of HLA-mismatches on most transplant outcomes(2–7). However, in our analysis, the degree of HLA incompatibility did not show any impact on OS, NRM, relapse, graft failure,or GvHD rates, even when vectorial HLA matching in the GvH or HvG directions was considered. A trend toward lower rates of aGVHD was found when HLA disparity was treated as a continuous variable but without any impact on all the other outcome parameters including OS.

Our findings are in agreement with a previous retrospectiveanalysis performed on 185 patients by Kasamon *et al*(35), which showed that greater HLA disparity according to the number of mismatched HLA-antigens in any direction (GvH or HvG) did not appear to influence the overall outcome after bone marrow transplantation with a high-dose post-transplant Cyclophosphamide.This analysis was confirmed when HLA disparity was assessed as either total HLA-mismatches or as mismatches according to specific vectors (mono-directional GvH or HvG HLA-mismatch). However, these data were generated in the context of a non-myeloablative HSCT platform; in contrast, all the patients included in this study received a myeloablative conditioning regimen. Furthermore, our platform of GVHD prophylaxis included

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cyclosporine A started at day 0 (instead of day +5), and PTCy was delivered at day +3 and +5 (rather than +3 and +4). In this study we didn't look for the possible contribution of individual HLA loci; indeed, the small number of patients with low mismatched and the absence of impact of the number of HLA-mismatch do not power our study for such analysis. Furthermore, the possible contribution of an individual locus may be different according to the remaining HLA disparity..

The lack of impact of different HLA disparity in our study is likely to not be specific for haploidentical donors, but rather to be embedded with the use of PTCy. Indeed, PTCy might level off HLA disparity in mismatched transplantation regardless of the type of donor, similarly to what already reported with other platforms exploited to overcome the HLA barrier in haploidentical HSCT (36-37). This effect of PTCy on GvHD prevention may be due to clonal deletion of allo-reactive T cells activating at time of transplantation and possibly to the preservation of T-regulatory cells, eventually shaping post-transplant immune reconstitution (38). In our cohort, the incidence of relapse was less than 30% at 3 years, again with no impact of HLA disparity; thus, these results may suggest that the graft *versus* leukemia (GvL) effect is spared, possibly together with anti-infectious protection (in our study, the rate of infectious complication was not increased; data not shown).

In conclusion, these data support the idea that PTCy exhibits the property of overcoming the HLA barrier; however, residual GvHD may also develop through non-HLA antigens, such as minor histocompatibility antigens. It remains to be determine whether this anti-GvHD effect of PTCy may be extended to the context of other HSCT settings. Prospective clinical trials are needed to adequately investigate the possible impact of PTCy in the context of unrelated HSCT, or even HLA-identical related transplantations.

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Figure 1a: Kaplan & Meier probability of overall survival after myeloablative conditioning. Low mismatch *vs*. high mismatch. p= 0.58

Figure 1b: Cumulative incidence of non-relapse mortality after myeloablative conditioning. Low mismatch *vs*. high mismatch. p= 0.93

Figure 2a: Incidence of grade II-IV acuteGvHD after myeloablative conditioning. Low mismatch *vs*. high mismatch. p= 0.13

Figure 2b: Cumulative incidence of moderate-severe chronic GvHD after myeloablative conditioning. Low mismatch *vs*. high mismatch. p= 0.84

Figure 3: Cumulative incidence of relapse. Low mismatch *vs*. high mismatch after myeloablative conditioning. p= 0.26

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Table 1. Clinical characteristics of patients (numbers in bracket indicate %; ± indicates standard deviation. **: p=0.004;

Demographic and clinical characteristics	**Allpatients (N = 318)**	**Low mm (0-2) (n=22)**	**High mm (3-4) (n=296)**	**Low mm GvH (0-2) (n=54)**	**High mm GvH (3-4) (n=264)**	**Low mm HvG (0-2) (n=46)**	**High mm HvG (3-4) (n=272)**
Male gender, n (%)	172 (54.1)	14 (63.6)	158 (53.3)	33 (61.1)	139 (52.7)	24 (52.2)	148 (54.4)
Age	48 ± 14.9	51.4 ± 12.7	47.7 ± 15	51.5 ± 11.2	47.3 ± 15.4	49.6 ± 13.2	47.7 ± 15.1
Male gender donor n(%)	193 (60.7)	13 (59.1)	180 (60.8)	37 (68.5)	156 (59.1)	28 (60.9)	165 (60.7)
Donor mother	27 (8.5)	1 (4.6)	26 (8.8)	2 (3.7)	25 (9.5)	3 (6.5)	24 (8.8)
Disease							
- AML	130 (40.9)	8 (36.4)	122 (41.2)	16 (29.6)	114 (43.2)	16 (34.7)	114 (41.9)
- ALL	64 (20.1)	2 (9.1)	62 (21)	4 (7.4)	60 (22.7)	8 (17.4)	56 (20.6)
- NHL	20 (6.3)	3 (13.6)	17 (5.8)	8 (14.8)	12 (4.5)	4 (8.7)	16 (5.9)
- CLL	10 (3.1)	1 (4.6)	9 (3)	4 (7.4)	6 (2.3)	2 (4.4)	8 (2.9)
- MM	13 (4.1)	2 (9.1)	11 (3.7)	3 (5.6)	10 (3.8)	2 (4.4)	11 (4.1)
- MPD	48 (15.1)	3 (13.6)	45 (15.2)	13 (24.1)	35 (13.3)	6 (13)	42 (15.4)
- MDS	33 (10.4)	3 (13.6)	30 (10.1)	6 (11.1)	27 (10.2)	8 (17.4)	25 (9.2)
Active disease at BMT	144 (45.3)	12 (54.6)	132 (44.6)	34 (63)	**110 (41.7)****	21 (45.7)	123 (45.2)
TNC infused 1-unit incr.	3.38 ± 1.16	3.55 ± 1.31	3.36 ± 1.15	3.49 ± 1.17	3.35 ± 1.16	3.3 ± 1.2	3.4 ± 1.1
AB0 major incompatibility	62 (19.5)	5 (22.7)	57 (19.3)	9 (16.7)	53 (20.1)	10 (21.7)	52 (19.1)
CMV serology (don/rec)							
+/+	200 (62.9)	14 (63.6)	186 (62.8)	34 (63)	166 (62.9)	30 (65.2)	170 (62.5)
+/-	20 (6.3)	1 (4.5)	19 (6.4)	2 (3.7)	18 (6.8)	4 (8.7)	16 (5.9)
-/+	73 (23)	6 (27.3)	67 (22.7)	15 (27.8)	58 (22)	8 (17.4)	65 (23.9)
-/-	25 (7.8)	1 (4.6)	24 (8.1)	3 (5.5)	22 (8.3)	4 (8.7)	21 (7.7)
Conditioning regimen							

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TT FluBu (2 days)	120 (37.6)	11 (50)	109 (36.8)	23 (42.6)	97 (36.7)	18 (39.1)	102 (37.5)
TT FluBu (3 days)	125 (39.3)	7 (31.8)	118 (39.9)	21 (38.9)	104 (39.4)	20 (43.5)	105 (38.6)
TBI 330x3 Flu	38 (12)	0 (0)	38 (12.8)	5 (9.3)	33 (12.5)	2 (4.4)	36 (13.2)
TBI 200x2 Flu	18 (5.7)	1 (4.6)	17 (5.8)	2 (3.7)	16 (6.1)	3 (6.5)	15 (5.5)
TT Flu Mel	17 (5.4)	3 (13.6)	14 (4.7)	3 (5.5)	14 (5.3)	3 (6.5)	14 (5.2)

Abbreviations: mm=mismatched;AML=acute myeloidleukemia; ALL=acute lymphoblasticleukemia; NHL=non-Hodgkin'slymphoma; CLL=chroniclymphocyticleukemia; MM= multiple myeloma; MPD=myeloproliferative disease; MDS=myelodysplasticsyndrome; TNC=totalnucleatedcells; CMV=citomegalovirus; TT=thyotepa; Flu=fludarabine; Bu=busulfan; TBI=total body irradiation; Mel=melphalan

Table 2. HLA mismatches

Number of HLA mismatched	**Total HLA mismatched** # (%)	**HLA mismatched GVH direction**	**HLA mismatched HVG direction**
4 antigen	231 (73%)	164 (52%)	185 (58%)
3 antigen	65 (20%)	100 (32%)	87 (28%)
2 antigen	14 (4%)	36 (11%)	33 (10%)
1 antigen	5 (2%)	10 (3%)	6 (2%)
0 antigen	3 (1%)	8 (2%)	7 (2%)

Abbreviations: GVH=graft vs host; HVG=host vs graft

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Table 3. Univariate analysis of OS and NRM in relation to HLA mismatched and other transplant variables.

	OS		NRM	
Demographic and clinical characteristics	**HR (95% CI)**	**p-value**	**SHR (95% CI)**	**p-value**
Total Mismatch (3-4 vs 0-1-2)	0.83 (0.44-1.59)	0.58	1.05 (0.38-2.93)	0.93
Bidirectional Mismatch (4 vs 0-1-2-3)	0.99 (0.70-1.41)	0.96	1.29 (0.77-2.13)	0.33
Total Mismatch, continuous	1.01 (0.79-1.29)	0.95	1.06 (0.72-1.54)	0.78
Gender, Male	1.04 (0.74-1.46)	0.83	1.03 (0.62-1.71)	0.92
Age, 1-year increase	1.01 (0.99-1.02)	0.092	1.03 (1.01-1.05)	***0.002***
Gender donor, Male	1.06 (0.74-1.50)	0.76	0.96 (0.57-1.60)	0.87
Donor mother	0.77 (0.40-1.46)	0.42	0.16 (0.02-1.17)	0.071
Disease		0.58		0.11
- AML	1.00 (ref)		1.00 (ref)	
- ALL	0.97 (0.61-1.56)		0.41 (0.17-0.97)	
- NHL	1.18 (0.60-2.31)		0.67 (0.20-2.27)	
- CLL	1.04 (0.38-2.87)		0.89 (0.21-3.86)	
- MM	1.12 (0.45-2.80)		0.76 (0.18-3.30)	
- MPD	1.35 (0.83-2.18)		1.58 (0.84-2.96)	
- MDS	0.63 (0.32-1.24)		0.52 (0.19-1.45)	
Active disease at transplant	3.61 (2.50-5.21)	***<0.001***	2.44 (1.44-4.15)	***<0.001***
TNC infused 1-unit increase	1.01 (0.88-1.17)	0.88	0.91 (0.73-1.13)	0.38
AB0 major incompatibility	1.19 (0.79-1.79)	0.42	1.13 (0.61-2.07)	0.70
CMV serology (donor/recipient)		0.53		0.17
+/+	1.00 (ref)		1.00 (ref)	
+/-	0.86 (0.41-1.77)		0.63 (0.20-1.98)	
-/+	0.99 (0.66-1.50)		0.64 (0.34-1.23)	
-/-	0.59 (0.27-1.28)		0.16 (0.02-1.21)	
Conditioning regimen		0.12		0.18
TT Flu Bu (2 days)	1.00 (ref)		1.00 (ref)	
TT Flu Bu (3 days)	0.78 (0.52-1.16)		0.88 (0.50-1.54)	
TBI 330x3 Flu	1.06 (0.64-1.76)		0.45 (0.16-1.30)	
TBI 200x2 Flu	0.55 (0.22-1.39)		0.51 (0.12-2.10)	
TT Flu Mel	1.81 (0.92-3.57)		1.93 (0.80-4.69)	

Abbreviations: AML=acute myeloidleukemia; ALL=acute lymphoblasticleukemia; NHL=non-Hodgkin'slymphoma; CLL=chroniclymphocyticleukemia; MM= multiple myeloma; MPD=myeloproliferative disease; MDS=myelodysplasticsyndrome;

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TNC=totalnucleatedcells; CMV=citomegalovirus; TT=thyotepa; Flu=fludarabine; Bu=busulfan; TBI=total body irradiation; Mel=melphalan

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Tab 4. Multivariate analysis of OS, NRM, relapse and graft rejection.

	Variable*		HR (95%CI)	p
OS				
	Active disease at transplant		3.61 (2.50-5.21)	<0.001
NRM				
	Age, 1-year increase		1.02 (1.00-1.04)	0.024
	Active diseaseattransplant		2.03 (1.18-3.50)	0.011
Relapse				
	Active diseaseattransplant		2.86 (1.87-4.38)	<0.001
	Conditioning regimen			0.035
		TT Flu Bu (2 days)	1.00 (ref)	
		TT Flu Bu (3 days)	1.16 (0.71-1.89)	
		TBI 330x3 Flu	2.47 (1.36-4.47)	***
		TBI 200x2 Flu	0.79 (0.25-2.49)	
		TT Flu Mel	1.44 (0.71-2.94)	
GraftRejection				
	Gender patient, Male		0.41 (0.16-1.07)	0.067
	Conditioning regimen			0.042
		TT Flu Bu (2 days)	1.00 (ref)	
		TT Flu Bu (3 days)	0.18 (0.05-0.66)	
		TBI 330x3 Flu	0.21 (0.03-1.65)	
		TBI 200x2 Flu	NE	
		TT Flu Mel	0.60 (0.12-3.07)	
	Active disease at transplant		2.79 (1.00-7.85)	0.05

*All variables with a p-value <=0.15 at univariable analysis were considered for the multivariable model. Only those significant were entered into the final multivariable model.

Abbreviations: TT=thyotepa; Flu=fludarabine; Bu=busulfan; TBI=total body irradiation; Mel=melphalan.

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Table 5. Univariate analysis of aGVHD, cGVHD and Relapse in relation to HLA mismatched (GvH direction) and other transplant variables.

	aGVHD		cGVHD		Relapse	
Demographic and clinical characteristics	**SHR (95% CI)**	**p-value**	**SHR (95% CI)**	**p-value**	**SHR (95% CI)**	**p-value**
Mismatch GVH (3-4 vs 0-1-2)	2.02 (0.81-5.08)	0.13	1.11 (0.42-2.94)	0.84	0.74 (0.44-1.25)	0.26
Mismatch GvH (4 vs 0-1-2-3)	1.37 (0.81-2.34)	0.24	0.80 (0.42-1.53)	0.51	0.92 (0.61-1.38)	0.69
Total Mismatch GVH, continuous	1.35 (0.98-1.87)	0.065	0.96 (0.68-1.34)	0.81	0.91 (0.74-1.13)	0.41
Gender, Male	0.89 (0.53-1.51)	0.67	1.27 (0.66-2.44)	0.47	1.03 (0.69-1.54)	0.89
Age, 1-year increase	1.01 (0.99-1.03)	0.22	1.00 (0.98-1.02)	0.81	0.99 (0.98-1.01)	0.25
Gender donor, Male	0.81 (0.47-1.38)	0.44	0.77 (0.40-1.46)	0.42	1.14 (0.76-1.73)	0.53
Donor mother	0.85 (0.30-2.40)	0.76	0.90 (0.28-2.89)	0.85	1.28 (0.68-2.43)	0.45
Disease		0.94		0.55		0.14
- AML	1.00 (ref)		1.00 (ref)		1.00 (ref)	
- ALL	1.03 (0.51-2.08)		1.36 (0.58-3.15)		1.85 (1.08-3.16)	
- NHL	0.51 (0.12-2.24)		0.54 (0.08-3.86)		1.50 (0.66-3.42)	
- CLL	1.81 (0.37-8.98)		Notestimable		1.91 (0.62-5.90)	
- MM	0.83 (0.20-3.45)		1.67 (0.40-6.99)		2.25 (1.02-4.95)	
- MPD	1.06 (0.50-2.25)		1.19 (0.41-3.43)		1.22 (0.66-2.28)	
- MDS	0.82 (0.32-2.15)		2.04 (0.82-5.09)		0.83 (0.37-1.83)	
Active disease at transplant	0.91 (0.53-1.54)	0.71	1.20 (0.63-2.31)	0.58	2.68 (1.76-4.07)	***<0.001***
TNC infused 1-unit increase	1.01 (0.78-1.30)	0.95	1.04 (0.79-1.36)	0.79	1.04 (0.86-1.25)	0.70
AB0 major incompatibility	1.29 (0.72-2.34)	0.38	1.32 (0.62-2.80)	0.48	1.24 (0.76-2.03)	0.39
CMV serology (donor/recipient)		0.39		0.14		0.45
+/+	1.00 (ref)		1.00 (ref)		1.00 (ref)	
+/-	1.22 (0.48-3.06)		0.62 (0.15-2.63)		1.28 (0.61-2.69)	
-/+	0.53 (0.24-1.18)		0.25 (0.07-0.83)		1.42 (0.89-2.27)	
-/-	1.16 (0.49-2.75)		0.67 (0.21-2.14)		0.88 (0.38-2.07)	
Conditioning regimen		0.98		0.89		***0.05***
TT Flu Bu (2 days)	1.00 (ref)		1.00 (ref)		1.00 (ref)	
TT Flu Bu (3 days)	1.01 (0.55-1.86)		1.20 (0.56-2.56)		1.02 (0.63-1.67)	
TBI 330x3 Flu	1.15 (0.49-2.70)		1.36 (0.48-3.85)		1.89 (1.06-3.37)	
TBI 200x2 Flu	1.27 (0.44-3.68)		1.72 (0.50-5.87)		0.60 (0.18-1.97)	
TT Flu Mel	1.26 (0.38-4.22)		1.63 (0.39-6.94)		1.96 (0.95-4.02)	

Abbreviations: AML=acute myeloidleukemia; ALL=acute lymphoblasticleukemia; NHL=non-Hodgkin'slymphoma; CLL=chroniclymphocyticleukemia; MM= multiple myeloma; MPD=myeloproliferative disease; MDS=myelodysplasticsyndrome;

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TNC=totalnucleatedcells; CMV=citomegalovirus; TT=thyotepa; Flu=fludarabine; Bu=busulfan; TBI=total body irradiation; Mel=melphalan

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Table 6 Univariate analysis of graft rejection in relation to HLA mismatched (HvG) and other transplant variables.

	Graftrejection	
Demographic and clinical characteristics	**OR (95% CI)**	**p-value**
Mismatch HGV (3-4 vs 0-1-2)	1.02 (0.29-3.60)	0.98
Mismatch HGV (4 vs 0-1-2-3)	1.47 (0.58-3.76)	0.42
Total Mismatch, continuous	0.95 (0.59-1.52)	0.83
Gender, Male	0.50 (0.20-1.24)	0.13
Age, 1-year increase	1.05 (1.01-1.09)	***0.022***
Gender donor, Male	2.17 (0.77-6.08)	0.12
Donor age, 1 year increase	0.99(0.96 – 1.03)	0.75
Donor mother	1.15 (0.25-5.20)	0.86
Disease		0.21
- AML	1.00 (ref)	
- ALL	0.57 (0.11-2.81)	
- LNH	NE	
- CLL	4.39 (0.78-24.69)	
- MM	3.19 (0.59-17.28)	
- MPD	2.51 (0.80-7.89)	
- MDS	1.13 (0.22-5.73)	
Active disease at transplant	3.26 (1.23-8.62)	***0.018***
TNC infused 1-unit increase	0.84 (0.56-1.28)	0.42
AB0 major incompatibility	0.42 (0.09-1.83)	0.25
CMV serology (donor/recipient)		0.14
+/+	1.00 (ref)	
+/-	0.53 (0.07-4.21)	
-/+	0.28 (0.06-1.26)	
-/-	NE	
Conditioning regimen		***0.0078***
TT Flu Bu (2 days)	1.00 (ref)	
TT Flu Bu (3 days)	0.17 (0.05-0.61)	
TBI 330x3 Flu	0.19 (0.02-1.48)	
TBI 200x2 Flu	NE	
TT Flu Mel	0.93 (0.19-4.49)	

Abbreviations: AML=acute myeloidleukemia; ALL=acute lymphoblasticleukemia; NHL=non-Hodgkin'slymphoma; CLL=chroniclymphocyticleukemia; MM= multiple

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myeloma; MPD=myeloproliferative disease; MDS=myelodysplasticsyndrome; TNC=totalnucleatedcells; CMV=citomegalovirus; NE = notevaluable; TT=thyotepa; Flu=fludarabine; Bu=busulfan; TBI=total body irradiation; Mel=melphalan

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