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ORIGINAL ARTICLE

KIR-ligand incompatibility in the graft-*versus*-host direction improves outcomes after umbilical cord blood transplantation for acute leukemia

R Willemze[1,2], CA Rodrigues[1], M Labopin[3], G Sanz[4], G Michel[5], G Socié[6], B Rio[7], A Sirvent[8], M Renaud[9], L Madero[10], M Mohty[11], C Ferra[12], F Garnier[1,13], P Loiseau[6], J Garcia[14], L Lecchi[15], G Kögler[16], Y Beguin[17], C Navarrete[18], T Devos[19], I Ionescu[1], K Boudjedir[1], A-L Herr[1], E Gluckman[1] and V Rocha[1,3,6] on behalf of Eurocord-Netcord and Acute Leukaemia Working Party of the EBMT, Paris (F)[20]

[1]Clinical Research Unit, Eurocord Office, APHP, Hôpital Saint Louis, Université de Paris VII, Paris, France; [2]Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands; [3]Acute Leukemia Working Party, EBMT-Paris Office, Hôpital Saint Antoine AP-HP, Université Pierre et Marie Curie Paris 6, Paris, France; [4]Servicio de Hematologie, Hospital Universitario La Fe, Valencia, Spain; [5]Service d'Oncologie Pédiatrique, Hôpital d'Enfants de la Timone, Marseille, France; [6]Department of Hematology and Bone Marrow Transplantation, Hôpital Saint Louis and Université de Paris VII, Denis Diderot, Paris, France; [7]Service d'Hématologie, Hôtel Dieu, Paris, France; [8]Hématologie Clinique, Hôpital de l'Archet 1, Nice, France; [9]Department of Hematology, Hôpital La Miletrie, Poitiers, France; [10]Department of Pediatrics, Nino Jesus Children's Hospital, Madrid, Spain; [11]Department of Hematology, Hôtel Dieu, Nantes, France; [12]Department of Hematology, Hospital Universitari Germans Trias I Pujol, Barcelona, Spain; [13]French Network of Cord Blood Banks, Paris, France; [14]Cord Blood Bank, Barcelona, Spain; [15]Cord Blood Bank and GRACE, Milano, Italy; [16]Eurocord José Carreras Stammzellbank, Düsseldorf, Germany; [17]Cord Blood Bank, Liège, Belgium; [18]NHS Cord Blood Bank, London, UK and [19]De Leuvense Navelstrengbloedbank, Leuven, Belgium

Donor killer cell immunoglobulin-like receptor (KIR)-ligand incompatibility is associated with decreased relapse incidence (RI) and improved leukemia-free survival (LFS) after haploidentical and HLA-mismatched unrelated hematopoietic stem cell transplantation. We assessed outcomes of 218 patients with acute myeloid leukemia (AML $n=94$) or acute lymphoblastic leukemia ($n=124$) in complete remission (CR) who had received a single-unit unrelated cord blood transplant (UCBT) from a KIR-ligand-compatible or -incompatible donor. Grafts were HLA-A, -B or -DRB1 matched ($n=21$) or mismatched ($n=197$). Patients and donors were categorized according to their degree of KIR-ligand compatibility in the graft-versus-host direction by determining whether or not they expressed HLA-C group 1 or 2, HLA-Bw4 or HLA-A3/-A11. Both HLA-C/-B KIR-ligand- and HLA-A-A3/-A11 KIR-ligand-incompatible UCBT showed a trend to improved LFS ($P=0.09$ and $P=0.13$, respectively). Sixty-nine donor–patient pairs were HLA-A, -B or -C KIR-ligand incompatible and 149 compatible. KIR-ligand-incompatible UCBT showed improved LFS (hazards ratio = 2.05, $P=0.0016$) and overall survival (OS) (hazards ratio = 2.0, $P=0.004$) and decreased RI (hazards ratio = 0.53, $P=0.05$). These results were more evident for AML transplant recipients (2-year LFS and RI with or without KIR-ligand incompatibility 73 versus 38% ($P=0.012$), and 5 versus 36% ($P=0.005$), respectively). UCBT for acute leukemia in CR from KIR-ligand-incompatible donors is associated with decreased RI and improved LFS and OS.
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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) from family, unrelated bone marrow or cord blood donors is a curative treatment for a considerable number of patients with acute leukemia. Major histocompatibility complex-restricted donor T cells play an important role in HLA-matched as well as HLA-mismatched HSCT by recognizing differences in minor and major histocompatibility antigens between donor and recipient.[1,2] A novel mechanism that relies on interactions between donor natural killer (NK) cells and recipient cells was demonstrated in the setting of haploidentical family donor HSCT.[3–5] NK cells express on their surface inhibitory killer cell immunoglobulin-like receptors (KIRs) that recognize allotypic determinants ('KIR ligands') shared by certain HLA class I allele groups. KIR2DL1 recognizes HLA-C alleles with a Lys^{80} residue (HLA-Cw4 and related, 'group 2' alleles), KIR2DL2 and KIR2DL3 recognize HLA-C with an Asn^{80} residue (HLA-Cw3 and related, 'group 1' alleles); KIR3DL1 is the receptor for HLA-B alleles sharing the Bw4 supertypic specificity.[3–5] Finally, KIR3DL2 was shown to function as a receptor for HLA-A3/-A11 alleles when bound to Epstein–Barr virus (EBV) peptides.[6]

The role of KIR and KIR ligand in the context of HSCT has been investigated over the past 10 years. In transplants that are KIR-ligand mismatched in the graft-versus-host (GvH) direction, donor NK cells expressing inhibitory KIRs, which do not recognize ligand(s) on recipient targets, are released from HLA inhibition and mediate alloreactions leading to clinically significant graft-versus-leukemia effects. Clinical studies in haploidentical[7–9] and in some (un)related donor transplants[10–12] showed that KIR-ligand incompatibilities in the GvH direction are associated with a decreased incidence of relapse and improved disease-free and overall survival (OS) after HSCT. Several unrelated donor transplant studies did not, however, report any effect.[13–17]

Umbilical cord blood units from unrelated donors constitute another well-established source of stem cells for HSCT in patients with leukemias. The main advantages are rapid availability and the possibility to perform HLA-mismatched transplantations due to a relatively low risk of severe GvH disease (GvHD)[18–21] that increases considerably the probability of finding a suitable donor. Moreover, outcomes after umbilical cord blood transplants (UCBTs) are comparable with those after

Correspondence: Professor R Willemze, Department of Hematology, Leiden University Medical Center, Albinusdreef 2, PO Box 9600, Leiden 2300 RC, The Netherlands.
E-mail: r.willemze@lumc.nl
[20]Other participating authors are listed in Acknowledgements.
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unrelated bone marrow transplantation in children and adults with acute leukemias.[19,21]

We hypothesized that in the setting of HLA-mismatched UCBT, NK-cell alloreactivity might come into play and influence outcomes. From the Eurocord database we retrieved all patients who had received a single-unit UCBT for acute leukemia in complete remission (CR). We grouped transplants according to the presence or absence of KIR-ligand mismatches that were shown to trigger donor-versus-recipient NK-cell alloreactivity in haploidentical transplantation, that is mismatches for HLA-C group 1, HLA-C group 2 and the HLA-Bw4 allele group. Moreover, as HLA-A3/-A11 KIR-ligand mismatches were not encountered in haploidentical transplants, we decided to include them in our evaluation. As HLA-A3/-A11 alleles were reported to function as KIR ligands when bound to EBV peptides, we hypothesized that, in view of the frequent EBV reactivation after transplantation, EBV peptides were readily available for HLA-A3/-A11 binding, with the possible generation of HLA-A3/-A11-specific donor NK cells that were alloreactive against target cells from recipients lacking HLA-A3 and/or HLA-A11.

Materials and methods

Data collection

This retrospective analysis is based on data reported to the Eurocord Registry from European and non-European centers through a standardized questionnaire concerning patient, cord blood units, disease and graft characteristics, as well as transplant outcomes. The collected information was reviewed by two physicians and checked for computer-generated errors to ensure data quality.

All patients or their legal guardians gave informed consent for UCBT according to the Declaration of Helsinki. This study was approved by the Eurocord institutional review board.

Criteria for patient selection

The following criteria were required: (1) acute leukemia in CR, (2) HLA high-resolution typing for HLA-A, HLA-B, HLA-C and -DRB1 of patients and cord blood units, or HLA-A high-resolution typing for those cord blood units without complete high-resolution typing for HLA-B and/or HLA-C, (3) non-expanded, single, unrelated cord blood unit and (4) complete clinical data during at least 3 months of follow-up.

Inhibitory KIR-ligand assessment in recipients and donors

As of 1 October 2007, 218 patient–donor pairs met the eligibility criteria. Complete information on high-resolution typing for HLA-A, HLA-B, HLA-C and HLA-DRB1 was available for 218 patients and 198 donors, and sufficient information on high-resolution typing for HLA-A and either HLA-B or HLA-C was available for 20 donors to determine KIR-ligand (in)compatibility in the GvH direction.

High-resolution typing of recipient and donor HLA-B and HLA-C alleles was used to segregate patients and cord blood units in the following KIR-ligand groups as described earlier by Ruggeri *et al*.[22] HLA-C group 1 alleles; HLA-C group 2 alleles; and HLA-Bw4 group alleles. Non-HLA-Bw4 alleles were grouped in the HLA-Bw6 allele group, which is not a KIR ligand. In addition, patients and cord blood units were grouped according to whether or not they displayed HLA-A3/-A11 alleles, which are putative KIR ligands.[6]

Composition of the study group for KIR-ligand (in)compatibility in the GvH direction

HLA-C group 1 or 2 alleles, which were present in the cord blood units, were missing in 41 transplant recipients; HLA-Bw4 group alleles were missing in 12 and HLA-A3/-A11 in 22 patients. Combination of the data on HLA-A, HLA-B and HLA-C of donors and recipients resulted in 69 (32%) donor–patient pairs with KIR-ligand incompatibility in the GvH direction. Fifty-one patients were HLA-C, group 1 or 2, 19 patients HLA-Bw4 and 18 patients HLA-A3/-A11 KIR-ligand mismatched in the host-versus-graft direction with the donor. Pairs in which the donor did not miss a ligand in the patient but did not possess a KIR ligand present in the recipient ($n = 50$) were pooled with the 99 KIR-ligand-matched pairs and, for the purpose of data analyses, are henceforth referred to as the KIR-ligand-compatible or -matched group, which comprised a total of 149 (68%).

End points

The main end points were leukemia-free survival (LFS), which was defined as the time from transplantation to either first relapse or death in CR; relapse incidence (RI), defined as any morphologically proven recurrence of leukemia occurring after the allograft; and OS, defined as the time from transplantation until the date of death. Patients were censored at the time of relapse or of the last follow-up.

Other end points were engraftment, non-relapse mortality (NRM) and acute or chronic GvHD. For engraftment, we considered the incidence of neutrophil recovery as defined by a neutrophil count of at least 0.5×10^9/l for three consecutive days and graft failure as no sign of neutrophil recovery as well as transient engraftment of donor cells within 60 days after transplantation. Chimerism data were available during the first 3 months after UCBT. Full donor chimerism was defined as the presence of more than 95% of the donor cells, mixed chimerism if more than 5% and less than 95% were donor cells and autologous recovery if less than 5% of cells were donor cells. Data on the methodology of chimerism detection were not available. Acute GvHD at day 100 was diagnosed and graded according to published criteria,[23] with histopathological confirmation when possible. NRM at 2 years was defined as deaths related to transplantation and not to relapse, and chronic GvHD at 2 years was diagnosed according to standard criteria[23,24] and evaluated in patients who survived at least 100 days with sustained engraftment.

Statistical analysis

Data were analyzed from the Eurocord database with the existing data as of October 2007. The duration of follow-up was the time to the last assessment for survivors. Variables related to the patient, to the disease, to the cord blood donor and to the transplant were compared (according to KIR-ligand compatibility status) using the χ^2 test for categorical and the Mann–Whitney test for continuous variables. All *P*-values are two-sided with type-1 error rate fixed at 0.05.

To analyze the outcome, we considered variables associated with the recipient (age, weight at time of transplantation, sex and cytomegalovirus (CMV) status), with the disease (acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), first, second or subsequent CR, and separately for ALL and

AML: good-, standard- or bad-risk cytogenetics), with the cord blood donor (HLA disparity, infused total nucleated cell and CD34+ cell doses) and with the transplant (year of transplant, reduced-intensity or myeloablative conditioning regimen, use of total body irradiation, use of ATG and GvHD prophylaxis). Cumulative incidence curves were used for RI and NRM in a competing risk setting, as death and relapse are competing together. Relapse and death in remission were considered as competing events to study neutrophil recovery, and acute and chronic GvHD.[25,26] Gray test was used for univariate comparisons.[27] Probabilities of LFS and OS were calculated using the Kaplan–Meier estimate; the two-sided log-rank test was used for univariate comparisons.

Factors associated with a *P*-value less than 0.10 by univariate analysis were included in multivariate analyses, using Cox proportional hazards for LFS and OS, and proportional subdistribution hazard regression model of Fine and Gray[28] for acute and chronic GvHD, neutrophil recovery, RI and NRM. Then a stepwise regression was performed using a threshold of 0.05. The diagnosis (AML and ALL) was added to the final model. All tests are two sided. The type I error rate was fixed at 0.05 for the determination of factors associated with time-to-event outcomes. Statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA) and S-Plus (MathSoft Inc., Seattle, WA, USA) software packages.

As the effects of KIR(-ligand) mismatching have been described to be associated with AML, a limited subgroup analysis was performed with respect to KIR-ligand incompatibility for patients with AML or ALL, separately.

Results

Patients, donor and transplant characteristics, and overall results of UCBT of the study group

The study group comprised 218 patients, undergoing a single-unit UCBT for AML or ALL in CR between 1997 and 2007 (median 2004). The median follow-up time is 14 months. The transplantations were performed in 57 centers. The cord blood units were supplied by 29 cord blood donor banks, mostly belonging to Netcord organization. Table 1 summarizes patient, donor and transplant characteristics for the patients transplanted with donors that were KIR-ligand incompatible ($n=69$) or KIR-ligand compatible ($n=149$) in the GvH direction. The diagnoses included ALL and AML, with good-, standard- or bad-risk cytogenetics in the first, second or third CR. The stages of both diseases were divided equally between the two groups. However, among patients with known cytogenetics, 17 AML patients out of 18 with a KIR-ligand-incompatible donor and 33 AML patients out of 57 with a KIR-ligand-compatible donor had bad-risk cytogenetics ($P=0.02$). CMV status was reported in 204 patients (111 positive) and in 202 cord blood donors (11 CMV positive). Combined information is available in 187 patient–donor pairs, of which 86 were both negative and 93 were patient positive/donor negative. EBV status of patients was reported in only 74 cases (34% of all patients) of which 58 were positive (78%) and in 78 donors (36% of all donors). CMV and EBV status were equally divided between the two KIR-ligand groups. The number of HLA disparities between donors and recipients was not significantly different between the two groups. Although a number of myeloablative and reduced-intensity conditioning regimens with or without ATG were used in the 57 participating transplant centers, the frequencies of myeloablative and reduced-intensity regimens and of use of ATG were not statistically different between the two KIR-ligand groups as well as other patient-, disease-, donor- and transplant-related factors (Table 1). Overall outcomes of the entire study group with respect to neutrophil engraftment, acute and chronic GvHD, non-relapse mortality, RI, LFS and OS are shown in Table 2.

Association of KIR-ligand incompatibility in the GvH direction with LFS, OS and RI

LFS and OS. To assess the impact of KIR-ligand mismatches that were shown to trigger donor-versus-recipient NK-cell alloreactivity in haploidentical transplantation, we determined LFS at 2 years for the donor–patient combinations that were mismatched (in the GvH direction) for HLA-C and/or HLA-Bw4 KIR ligands. It was 48 ± 9 versus $36\pm4\%$ ($P=0.09$) for those who were KIR-ligand matched.

As we had a relatively large group of 22 donor–recipient pairs that were mismatched for HLA-A3/-A11 (in the GvH direction), we were able to assess for the first time the role of this putative KIR ligand in transplantation outcomes; LFS was $62\pm11\%$ for the donor–patient combinations that were mismatched for HLA-A3/-A11 versus $35\pm4\%$ for those who were not A3/A11 mismatched ($P=0.13$). As both analyses demonstrated an increased LFS in an almost not overlapping fashion and as only six patients had two incompatibilities consisting of HLA-C group and/or HLA-Bw4 group incompatibilities in combination with an HLA-A3 and/or -A11 mismatch, we combined the two groups and performed a common statistical analysis.

Leukemia-free survival was significantly improved in patients receiving a UCBT from an HLA-A, -B or -C KIR-ligand incompatible donor compared with those who were transplanted with an HLA-A, -B or -C KIR-ligand compatible donor (in the GvH direction). The estimates for LFS at 2 years are 55 ± 7 and $31\pm4\%$ ($P=0.005$), respectively, for patients with and without donor KIR-ligand incompatibility in the GvH direction (Figure 1a).

Overall survival was also improved in patients receiving a UCBT from a KIR-ligand-incompatible donor compared with those who were transplanted with a KIR-ligand-compatible donor. The estimates for 2-year OS were 57 ± 7 and $40\pm4\%$ ($P=0.02$), respectively, for patients with and without donor KIR-ligand incompatibility in the GvH direction (Figure 1b).

In univariate analysis, which included age, sex, CMV status, diagnosis (AML or ALL), stage of the disease, infused total number of cells or of $CD34^+$ cells, HLA disparities, conditioning regimen, use of total body irradiation or ATG and KIR-ligand incompatibility (in the GvH direction), only <2 HLA disparity was associated with OS ($P=0.008$).

In multivariate analysis, KIR-ligand incompatibility was an independent risk factor associated with improved LFS and OS. Compatible HLA grafts or with one HLA disparity were also associated with improved OS (Table 3). The estimates for LFS at 2 years for AML and ALL were not significantly different. Within AML as well as ALL, LFS at 2 years was not different for the patients with good-, standard- or poor-risk cytogenetics (data not shown).

Relapse. The 2-year cumulative incidence of relapse was $32\pm4\%$ for the entire study group, and it was $37\pm4\%$ for the KIR-ligand-compatible group compared with $20\pm5\%$ for the KIR-ligand-incompatible group ($P=0.03$) (Figure 1c). In univariate analysis, other factors associated with RI were type of conditioning ($P=0.05$), use of total body irradiation ($P=0.08$) and ATG ($P=0.09$). In a multivariate analysis, myeloablative conditioning regimen (MAC) ($P=0.007$), two or more HLA disparities ($P=0.05$)

Table 1 Patient demographics according to the presence of a KIR-ligand-compatible and -incompatible cord blood donor in 218 UCBT for patients with acute leukemia in CR

	Entire population	*KIR-ligand-compatible donors*	*KIR-ligand-incompatible donors*	P-*value**
Number of patients	218	149	69	
Median age (years)	13.8 (0.5–69)	12.8 (0.6–69)	15 (0.5–64)	0.52
Year of transplant	2004 (97–07)	2004 (97–07)	2004 (98–07)	0.68
Median follow-up	14 months	13 months	15 months	
Median weight (kg)	49 (6.5–112)	47 (7–112)	50 (6.5–102)	0.52
Male, *n* (%)	122 (56)	84 (56)	38 (45)	0.86
Diagnosis, n *(%)*				0.27
AML	94 (43)	68 (46)	26 (38)	
ALL	124 (57)	81 (54)	43 (62)	
*Cytogenetics (*n = *174)*[a]				
AML good risk	5	5	0	
Standard risk	20	19	1	
Bad risk (%)	50 (67)	33 (58)	17 (94)	0.02
ALL good risk	11	8	3	
Standard risk	39	24	15	
Bad risk (%)	49 (49)	30 (48)	19 (51)	0.76
Status at transplant, n *(%)*				0.52
1st CR	105 (48)	69 (46)	36 (52)	
2nd CR	91 (42)	66 (44)	25 (36)	
>2nd CR	22 (10)	14 (10)	8 (12)	
TNC infused (median $\times 10^7$/kg)	3.09 (0.05–34.3)	3.14 (0.05–34.3)	2.86 (0.7–22.3)	0.29
CD34+ infused (median $\times 10^5$/kg)	1.31 (0.08–10.2)	1.37 (0.08–7.1)	1.2 (0.25–10.2)	0.22
Number of HLA disparities, n *(%)*[b]				0.27
6/6 match	21 (10)	16 (11)	5 (7)	
5/6 match	94 (43)	66 (44)	28 (41)	
4/6 match	93 (43)	57 (39)	36 (52)	
3/6 match	8 (3)	8 (5)	0	
2/6 match	2 (1)	2 (1)	0	
Conditioning regimen, n *(%)*				
RIC (*n* = 202)	35 (17)	25 (18)	10 (16)	0.77
TBI	122 (56)	80 (54)	42 (61)	0.35
Use of ATG/ALG (*n* = 196)	158 (81)	106 (79)	52 (84)	0.43
GvHD prophylaxis, n *(%)*				0.76
CsA	25 (11)	17 (12)	8 (12)	
CsA+prednisone	137 (63)	90 (60)	47 (68)	
CsA+MMF	34 (16)	25 (17)	9 (13)	
Other	22 (10)	17 (11)	5 (7)	

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG/ALG, antithymocyte/antilymphocyte globulin; CR, complete remission; CsA, cyclosporin; GvHD, graft-versus-host disease; MMF, mycophenolate mofetil; RIC, reduced-intensity conditioning; TBI, total body irradiation; TNC, total nucleated cells.

**P*-value represents the comparison of each characteristic according to the KIR-ligand group.

[a]Cytogenetic data available for 174 patients (AML, *n* = 75; ALL, *n* = 99): numbers and percentages of patients are given; ALL good risk: del(9p), t(12p)/t(12;21), t(10;14), t(14q11–q13), hyperdiploid; standard risk: normal cytogenetics and all remaining abnormalities; bad-risk cytogenetics: t(9;22), t(4;11), t(1;19), t(8;14), −7, +8, abn(11q23), hypodiploid/near triploid, complex abnormalities; AML good risk: t(8;21), inv.16 and t(15;17); standard risk: normal cytogenetics; bad risk: all other chromosomal abnormalities.

[b]On the basis of antigen-level HLA-A and HLA-B and allele-level HLA-DRB1 typing.

and KIR-ligand incompatibility ($P = 0.05$) were independently factors associated with a decreased risk of relapse (Table 3).

Association of KIR-ligand incompatibility in the GvH direction with engraftment, GvHD and NRM

Neutrophil recovery. Cumulative incidence of neutrophil recovery at day 60 for the entire group was 80 ± 3%, and it was 81 ± 4% for patients with and 79 ± 3% for those without KIR-ligand incompatibility, respectively ($P = 0.21$). The median time to neutrophil recovery was 21 days (4–40) for KIR-ligand-incompatible transplants and 25 (6–60) days for KIR-ligand-compatible transplants. In univariate analysis, the incidence of neutrophil recovery was associated with the median number of nucleated cells infused ($P = 0.015$) and HLA disparity ($P = 0.05$), but not with KIR-ligand incompatibility. In a multivariate analysis (Table 2), only a higher number of nucleated cells ($>3.09 \times 10^7$/kg) infused were associated with higher rates of neutrophil recovery ($P = 0.015$).

At 3 months after UCBT, chimerism data were available in 158 patients: 126 patients were full donors (80%), 15 had mixed chimerism (9%) and 17 (10%) had autologous reconstitution. There was no association between KIR-ligand incompatibility and full donor chimerism ($P = 0.40$).

Acute and chronic GvHD. Sixty-five patients presented grade II–IV acute GvHD (grade II, $n=36$, 18%; grade III, $n=14$, 7%; grade IV, $n=15$, 7%). At day 100, cumulative incidence of grade II–IV acute GvHD for the entire population was $29\pm3\%$, and it was $28\pm5\%$ for patients with a KIR-ligand-incompatible donor and $30\pm3\%$ for patients with a KIR-ligand-compatible donor (in the GvHD direction), respectively ($P=0.82$). The 2-year cumulative incidence of chronic GvHD was 19% ($n=29$ for 171 patients at risk). There was no association of chronic GvHD with the KIR-ligand groups.

NRM and non-relapse causes of death. The 2-year cumulative incidence of NRM was $30\pm3\%$, and it was $31\pm4\%$ for patients with a KIR-ligand-compatible donor and $25\pm5\%$ for those with a KIR-ligand-incompatible donor ($P=0.34$). In a multivariate analysis, only RIC and a lower number of HLA disparities (compatible or one HLA disparity) were associated with decreased NRM (Table 3). Death due to opportunistic infections (viral, fungal and parasitic) was more frequent in the KIR-ligand-compatible donor group than in the KIR-ligand-incompatible donor group (Table 4). Unfortunately, more detailed information on the nature of these lethal infections is not available in the Eurocord database, but they occurred equally in the groups of CMV-positive and CMV-negative patients.

Table 2 Overall outcomes after UCBT of 218 patients with acute leukemia in CR

Outcomes	*No. of events/patients at risk*	*Estimation*
Neutrophil recovery[a] at day 60	137/218	$80\pm3\%$
Acute GvHD[a] at day 100	65/205	$29\pm3\%$
Chronic GvHD[a] at 2 years	29/171	$19\pm3\%$
Relapse incidence[a] at 2 years	58/218	$32\pm4\%$
Non-relapse mortality[a] at 2 years	58/218	$30\pm3\%$
Leukemia-free survival[b] at 2 years	116/218	$38\pm4\%$
Overall survival[b] at 2 years	103/218	$45\pm4\%$

Abbreviation: GvHD, graft-versus-host disease; UCBT, umbilical cord blood transplant.
[a]Cumulative incidence function.
[b]Kaplan–Meier estimate.

Association of KIR-ligand incompatibility in the GvH direction with outcomes in patients with AML or ALL in CR

A limited subgroup analysis was performed for 94 patients with AML (47 patients in first CR; 40 patients in second CR and 7 patients in third CR) and 124 patients with ALL (58 patients in first CR, 51 patients in second CR and 15 patients in third CR).

In AML UCBT recipients, 2-year cumulative incidence of relapse was associated with the presence or absence of KIR-ligand incompatibility in the GvH direction (5 ± 4 versus $36\pm7\%$, $P=0.005$). The 2-year estimates for LFS were 73 ± 10 and $38\pm7\%$ ($P=0.012$) and for OS were 70 ± 11 and $36\pm8\%$ ($P=0.016$), respectively (Figure 2a).

In spite of the trend of a decreased RI and improved LFS and OS for those with a KIR-ligand-incompatible donor, there was not a significant statistical association in ALL patients (Figure 2b).

NRM and acute and chronic GvHD were not influenced by the diagnosis (AML or ALL) or by the KIR-ligand-compatibility status.

Discussion

This study demonstrates that inhibitory KIR-ligand incompatibility between donor and recipient in the GvH direction is

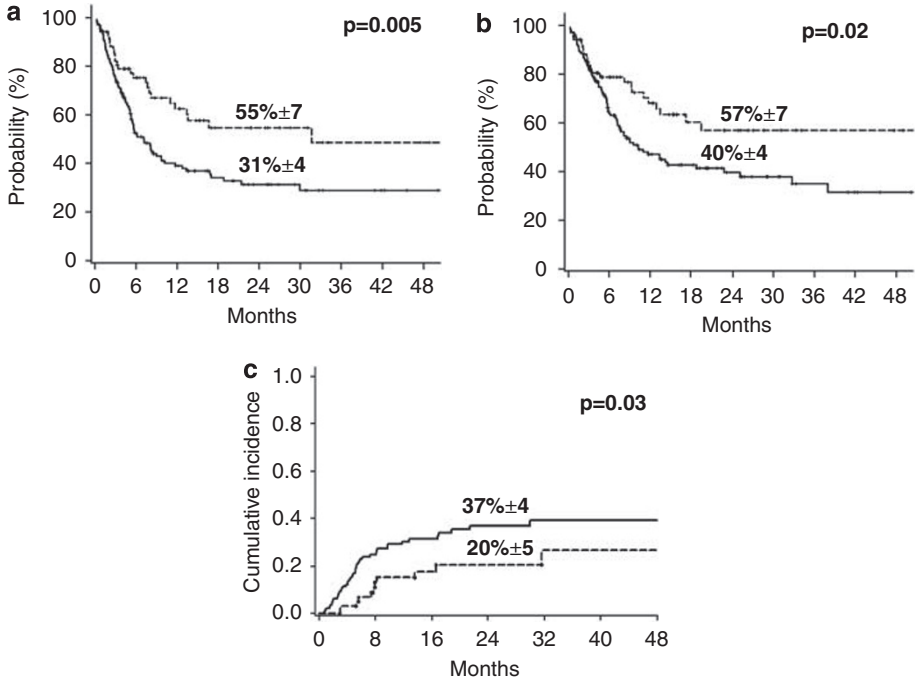

Figure 1 (**a**) Estimated leukemia-free survival in patients with Kir-ligand-compatible (———) ($n=149$) or -incompatible (- - - - - - -) donors ($n=69$), after UCBT for acute leukemia in CR. (**b**) Estimated overall survival in patients with Kir-ligand-compatible (———) ($n=149$) or -incompatible (- - - - - - -) donors ($n=69$), after UCBT for acute leukemia in CR. (**c**) Cumulative incidence of relapse in patients with Kir-ligand-compatible (———) ($n=149$) or -incompatible (- - - - - - - -) donors ($n=69$), after UCBT for acute leukemia in CR. CR, complete remission; UCBT, unrelated cord blood transplant.

Table 3 Multivariate analysis for overall survival, leukemia-free survival, relapse incidence, non-relapse mortality and neutrophil recovery

	Relative risk	*95% confidence interval*	P-*value*
Overall survival			
AML versus ALL	1.18	0.79–1.75	0.43
<2 HLA disparities[a]	1.79	1.2–2.7	0.004
Donor Kir-ligand incompatibility	2.00	1.24–3.22	0.004
Leukemia-free survival			
AML versus ALL	1.41	0.96–2.08	0.08
<2 HLA disparities	1.39	0.96–2	0.08
Donor Kir-ligand incompatibility	2.05	1.31–3.2	0.0016
Relapse incidence			
AML versus ALL	0.67	0.38–1.17	0.16
⩾2 HLA disparities	0.58	0.33–0.99	0.05
MAC regimens	0.42	0.22–0.79	0.007
Donor Kir-ligand incompatibility	0.53	0.28–0.99	0.05
Non-relapse mortality			
AML versus ALL	0.89	0.51–1.56	0.690
<2 HLA disparities	0.38	0.21–0.68	0.001
RIC regimens	0.12	0.03–0.51	0.004
Donor Kir-ligand incompatibility	0.6	0.31–1.16	0.13
Neutrophil recovery			
AML versus ALL	1.16	0.85–1.56	0.35
<2 HLA disparities	1.31	0.97–1.79	0.08
TNC $>3.09\times10^7$/kg	1.45	1.07–1.96	0.015

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; HLA, human leukocyte antigen; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; TNC, total nucleated cells infused.
[a]On the basis of antigen-level HLA-A and HLA-B and allele-level HLA-DRB1 typing.

Table 4 Causes of death according to the presence of a KIR-ligand-compatible and -incompatible cord blood donor in 218 UCBT for patients with acute leukemia in CR

	Entire population	*KIR-ligand-compatible donors*	*KIR-ligand-incompatible donors*	P-*value**
No. of patients	218	149	69	
Cause of death, n *(%)*				
Related to relapse	44 (45)	36 (46)	8 (35)	
Related to transplant	58 (55)	43 (54)	15 (65)	0.36
If transplant related (n)				
GvHD	11	6	5	
Interstitial pneumonitis	3	2	1	
VOD	2	2	0	
Hemorrhage	3	2	1	
Rejection	5	1	4	
Bacterial infection	5	3	2	
Viral infection	7	7	0	
LPD EBV	4	4	0	
Fungal infection	2	2	0	
Parasitic infection	1	1	0	
Cardiac toxicity	1	0	1	
ARDS	3	3	0	
Unknown	1	1	0	
Multiorgan failure	4	3	1	
Other	4	4	0	
Death due to opportunistic infections[a], *n* (%)	17 (30)	16 (38)	1 (7)	0.03[b]

Abbreviations: ARDS, acute respiratory distress syndrome; GvHD, graft-versus-host disease; LPD EBV, EBV-related lymphoproliferative disease; VOD, veno-occlusive disease.
**P*-value represents the comparison of each characteristic according to the KIR-ligand group.
[a]Among non-relapse-related deaths.
[b]Fisher's exact test.

associated with a decreased RI and an improved LFS and OS for patients with acute leukemia transplanted in CR with a single-unit unrelated umbilical cord blood graft. Multivariate analysis shows that these findings are independent of the number of HLA disparities between the donor and recipient.

We confirm our and other previous studies showing that a higher number of HLA disparities (two or more) are associated with diminished neutrophil recovery, increased NRM rates and decreased RI.[19,20] In contrast, KIR-ligand incompatibility was not associated with the cumulative incidence of neutrophil recovery within 60 days and NRM.

A limitation of our study is the short median follow-up (13 months for the KIR-ligand-matched and 15 months for the KIR-ligand-mismatched donor–patient pairs) due to the fact that HLA-C allelic typing has just recently been used for both umbilical cords and patients. However, the aim of our study was not to assess the actual risk of relapse after UCBT but to compare the incidence of relapse after UCBT with or without KIR-ligand mismatches in the GvH direction. We cannot rule out that a KIR-ligand-mismatched combination delays relapse, which would become evident after a longer follow-up.

Different effects of KIR/KIR-ligand incompatibilities on the outcome of allogeneic SCT have been reported. During the last 10 years, the understanding of NK-cell alloreactivity is rapidly expanding, and thus the methods, utilized through the years, to determine KIR mismatch, vary largely. Mismatches between donor KIR ligand and recipient KIR ligand in the GvH direction (the donor–recipient KIR-ligand model) have been used by the Perugia group and Hsu *et al.*,[7–9,12,22] between donor KIR and recipient KIR by Gagne *et al.*[28] and between donor KIR and recipient KIR ligand by Leung *et al.*[29,30]

Criticizing the donor–recipient KIR-ligand model one could argue that, as some donors might not have possessed the relevant *KIR* gene, a minority of donor–recipient pairs might have been misclassified in this study. In fact, donor-versus-recipient NK-cell alloreactivity was predicted on the basis of KIR-ligand mismatching without the direct assessment of the donor alloreactive NK-clone repertoire or donor KIR genotyping. However, it is worth noting that KIR genotyping is required

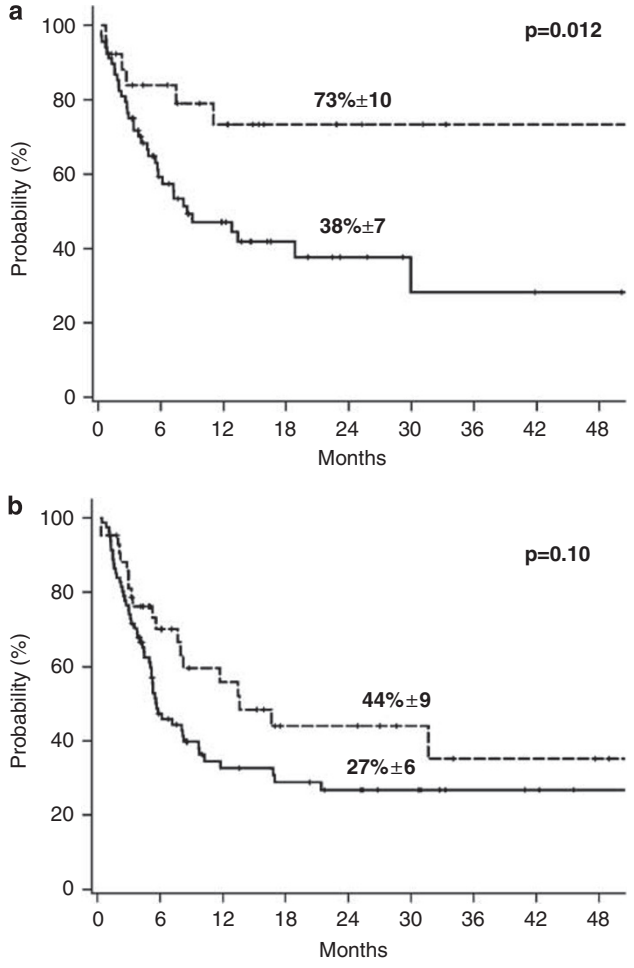

Figure 2 (**a**) Estimated leukemia-free survival in AML patients in CR with Kir-ligand-compatible (———) ($n=68$) or -incompatible (- - - - - - -) donors ($n=26$), after UCBT. (**b**) Estimated leukemia-free survival in ALL patients in CR with Kir-ligand-compatible (———) ($n=81$) or -incompatible (- - - - - - -) donors ($n=43$), after UCBT. AML, acute myeloid leukemia; CR, complete remission; UCBT, unrelated cord blood transplant.

in only a minority of donors as most possess a full complement of inhibitory *KIR* genes and have the potential to exert NK alloreactions. Moreover, the risk of misclassification arises when the donor does not possess/express the relevant KIR to exert alloreactivity. Under this circumstance, KIR-ligand mismatching mistakenly classifies a non-NK-alloreactive transplant pair as NK alloreactive. Therefore, in our series, such 'false positives' might have 'contaminated/diluted' the NK-alloreactive cohort. Thus, the only conceivable consequence of such misclassification is an underestimation of the effects of NK alloreactivity, which reinforces, rather than detracts from, our core observation that NK-cell alloreactivity favorably impacts on outcomes of cord blood transplantation.

Our findings in UCBT are in agreement with those of the Perugia group, which, using haploidentical family donor SCT, has shown that KIR-ligand incompatibility in the GvH direction is associated with a decreased incidence of graft failure, GvHD and relapse, and an improved survival of patients with AML.[7–9] They explain their clinical results by findings from mouse experiments and human *in vitro* data in which donor-versus-recipient alloreactive NK cells eliminate residual leukemic cells, ablate host-type dendritic cells responsible for triggering GvHD and attack residual host lymphohematopoietic cells, including T cells, that are responsible for graft rejection.[8] Our present analyses show that KIR-ligand incompatibility has a stronger effect on RI, LFS and OS in AML patients than in ALL patients. This finding is in accordance with clinical and *in vitro* data from Ruggeri *et al.*,[8] who showed that AML cells were killed *in vitro* by alloreactive NK clones, whereas only a sub-population of ALL cells was sensitive to NK-cell alloreactivity. As we observed a small but consistent KIR-ligand effect in our ALL patients, the data suggest that subgroups of ALL (T- or B-lineage ALL) may show differential sensitivities to donor NK-cell alloreactivity.

This study provides for the first time information on the role of HLA-A3/-A11 KIR-ligand mismatches in transplantation, which had been lacking until now, as they were not reported in haploidentical transplants. Surprisingly, HLA-A3/-A11 mismatching in the GvH direction emerged as just as good a predictor of favorable prognosis as HLA-C and HLA-Bw4 group mismatches. Our use of HLA-A3/-A11 mismatching as a predictor of NK-cell alloreactivity may be criticized, as the role of HLA-A3/-A11 as a ligand for KIR3DL2 is controversial. As HLA-A3/-A11 alleles were reported to function as KIR ligands when bound to EBV peptides,[6] it seems reasonable that, in view of frequent EBV reactivation after transplantation, EBV peptides were readily available for HLA-A3/-A11 binding, with the possible generation of HLA-A3/-A11-specific donor NK cells that were alloreactive against target cells from recipients lacking HLA-A3 and/or HLA-A11. In our data set, EBV status was reported in only 74 patients (36%), of which 58 (78%) were EBV positive. No data on EBV reactivation are available in the Eurocord database. Thus, the role of HLA-A3/-A11 as a KIR ligand cannot be further supported in this study.

This analysis shows that donor-versus-recipient NK-cell alloreactivity exerts a beneficial impact not only in haploidentical but also in cord blood transplantation. These two types of SCT have in common that the donors are highly HLA mismatched with the recipient, whereas after SCT, relatively little severe GvHD is observed. The post-transplant period is further characterized by a rapid recovery of NK cells and long-lasting T-cell immunoincompetence. As T cells in the cord blood graft are antigen inexperienced, they contain few or no cytotoxic T cells directed against viral and bacterial peptides, which may cross-react with HLA alloantigens. The relative lack of post-thymic T cells and the T-cell naivety may be responsible for the lower-than expected frequency of severe GvHD, even in the setting of one or two HLA mismatches, and for the delayed recovery of T-cell function after UCBT[31–33] to the same degree as after haploidentical SCT. Lack of memory T cells in the graft (due to T-cell depletion in haploidentical and T-cell naivety in cord blood transplants) apparently permits the recovery of fully functional NK cells. Interestingly, one study comparing T cell-depleted-versus-repleted unrelated donor transplants demonstrated that T cells in the graft adversely affected reconstitution of KIR-bearing NK cells and clinical outcomes.[34] Additional evidence that T cells antagonize reconstitution of potentially alloreactive, KIR-bearing NK cells derives from several other unrelated donor transplant studies, using T-cell-replete grafts.[10,11,13–15,33,35] Most studies showed no advantage in transplantation from KIR-ligand-mismatched donors,[13–15,30,31,36–38] whereas a few observed an increased graft-versus-leukemia effect.[10,11,39–41]

In haploidentical[29,30], matched sibling[12] and unrelated donor transplants,[36] the 'missing ligand' model was proposed as a powerful algorithm for predicting favorable transplant outcomes. It hypothesizes that alloreactions also occur when

KIR-ligand-matched donors possess KIR(s) for which neither donor nor recipient has an HLA ligand(s). These donors were shown to carry KIR-bearing NK cells in an anergic/regulated state.[42] The 'missing ligand' model proposes that, upon transfer into the recipient, they may become activated and exert a graft-versus-leukemia effect. However, in the UCBT setting, as in a recent haploidentical transplant study,[9] no informative results emerged when we analyzed the outcomes of 'missing ligand' transplants (data not shown).

In conclusion, in patients with acute leukemia in CR, single-unit UCBT from donors who are KIR-ligand mismatched (for HLA-C group, HLA-Bw4 group and/or HLA-A3/-A11) in the GvH direction resulted in a lower incidence of relapse and in an improved LFS and OS as compared with UCBT from KIR-ligand-compatible donors. If these results are confirmed in a larger series of patients, KIR-ligand incompatibility in the GvH direction might be considered as a criterion for cord blood donor choice.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)