## Use of killer cell immunoglobulin-like receptor genes as early markers of hematopoietic chimerism after double-umbilical cord blood transplantation

It has been established that hematopoietic chimerism after double umbilical cord blood transplantation (dUCBT) can predict disease relapse and detect potential engraftment failure.[1-3] The use of a precise method to evaluate residual host hematopoiesis is imperative because it has been reported that the risk of relapse is higher in patients with mixed chimerism.[4] Up to now, real-time quantitative polymerase chain reaction (PCR) of single nucleotide polymorphism (SNP) markers remains the best method to accurately assess and quantify chimerism.[5,6] However, this method may be limited by the lack of available in-house SNP discriminant markers between both umbilical cord blood (UCB) units and patients and may not be reliable to assess early chimerism status after dUCBT. In this context, additional approaches are needed. Natural killer (NK) cells are the first population to reconstitute the patient's hematopoietic system following hematopoietic stem cell transplantation, before T lymphocytes,[7] and we previously reported that cord blood NK cells express killer cell immunoglobulin-like receptors (KIR).[8] Here, we investigated KIR genes as additional markers for early chimerism assessment after dUCBT. KIR gene content varies between individuals who can exhibit seven to fourteen inhibitory and activating KIR genes, defining multiple KIR genotypes. This broad KIR gene polymorphism should be useful as chimerism markers in the context of dUCBT.

In order to determine which UCB unit dominates engraftment, KIR genotyping was performed on 40 patients who underwent dUCBT at Nantes CHU, using a multiplex sequence-specific primer-PCR method[9] on day 90 after dUCBT and was compared to KIR genotyping in patients and in UCB units prior to dUCBT. The presence or absence of each KIR gene among 14 studied in patients, UCB units and recipients led us to determine the identity of cells reconstituting recipient hematopoiesis. As illustrated in Figure 1A, recipient #11 was characterized by the absence of *KIR2DL2* and *KIR2DS2* genes, which were present in this patient before dUCBT. Interestingly, *KIR2DL2* and *KIR2DS2* were absent in UCB1, but present in UCB2, highlighting that hematopoietic reconstitution resulted from only one UCB unit (unit 1) in this patient (Figure 1A). Phenotypic analysis of cord blood KIR$^+$ NK cells before dUCBT and recipient KIR$^+$ NK cells at day 60 after dUCBT using available anti-KIR monoclonal antibodies[16] allowed discrimination of inhibitory and activating KIR expression between the two UCB units (Figure 1B) and confirmed KIR genotyping results. In particular, UCB1 NK cells did not express KIR2DS1 and KIR2DL2, unlike NK cells from UCB2. Moreover, KIR3DL1 was only expressed on NK cells from UCB1. We observed that the recipient's KIR$^+$ NK cells on day 60 after dUCBT had the same KIR expression profile as NK cells from UCB1, with expression of KIR3DL1 and absence of KIR2DS1 and KIR2DL2, confirming engraftment of patient #11 with one full dominant UCB1.

Analysis of KIR genotyping also allows mixed chimerism to be detected, as illustrated in Figure 1C for patient #13 who presented the KIR2DL5A allele and the deleted non-expressed KIR2DS4 allele (i.e. 1D) before dUCBT. Both UCB units had the KIR2DL5B allele and expressed KIR2DS4. Recipient #13 had both A and B KIR2DL5 alleles and KIR2DS4, in particular, highlighting a mixed chimerism between the patient and one UCB unit. Although rare,[10] mixed chimerism between both UCB units may also be detected using KIR genotyping (*Online Supplementary Figure S1*). In several other cases, analysis of KIR genotype after dUCBT highlighted engraftment failure and autologous patient reconstitution, as illustrated in recipient #33 (Figure 1D).

KIR genotyping was performed in all recipients and was focused on discriminating KIR genes that allowed us to assess hematopoietic chimerism status by their presence or absence (*Online Supplementary Table S1*). Based on the calculated frequencies of each discriminating KIR gene, we highlight the major implication of three inhibitory and all activating KIR genes (Figure 2A). Overall, qualitative chimerism analysis at day 90 after dUCBT using KIR markers indicated full UCB unit reconstitution (60%), mixed patient/UCB unit reconstitution (20%) and autologous recovery (7.5%) in the recipients included in this study (Figure 2B). However, KIR genotyping did not allow chimerism status to be assigned in the five remaining dUCBT, which were therefore called "indeterminate" (Figure 2B). Although KIR genotyping is a reproducible and reliable method as initially reported,[9] even using a small amount of cord blood DNA (*Online Supplementary Figure S2*), the patients, UCB units and recipients had the same KIR genotype in these five cases, thus preventing any chimerism assessment using KIR markers. Moreover, KIR genotyping analysis of five other dUCBT revealed mixed chimerism, which was discordant with the conventional SNP analysis, being evaluated as "full donor chimerism" (n=4) or "patient reconstitution" (n=1) as illustrated for patient #15 (Figure 2C). Overall, 31 out of 40 dUCBT (3 dUCBT with patient reconstitution, 3 dUCBT with mixed chimerism and 25 dUCBT with full donor engraftment) had concordant results with the conventional SNP-based analysis (Figure 2D).

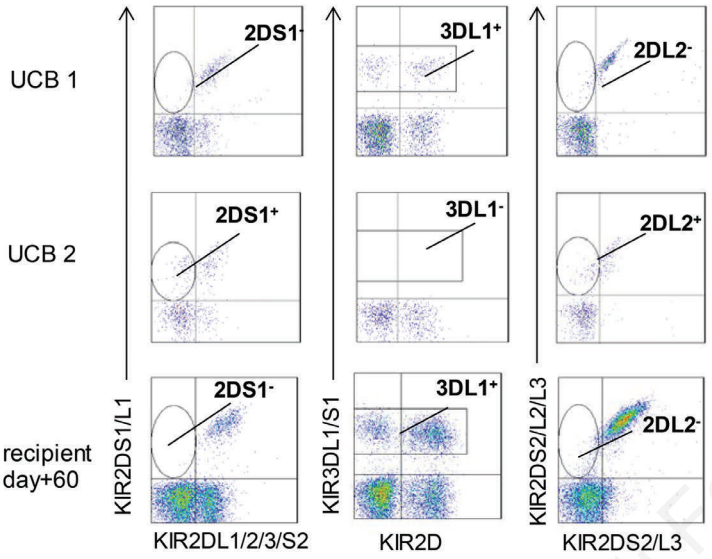
For some dUCBT with available DNA (n=6), we complemented the KIR genotyping with HLA class I allele typing to provide additional information since numerous HLA class I incompatibilities between UCB units and patients are frequently encountered in the context of dUCBT. Of note, HLA class I genes presenting large allelic polymorphisms are useful markers to re-assess the "indeterminate" dUCBT and the few discordant results between KIR genotyping and the SNP–based method. Our analysis based on KIR genotyping, with secondary complementary HLA class I allele typing, applied in a few cases, allowed chimerism status to be accurately determined in 100% of patients in full concordance with conventional approaches (Figure 2E). Indeed, as illustrated in Figure 2F, KIR genotyping of recipient #39 on day 90 did not allow chimerism status to be assigned. However, using HLA-B typing on day 90 this recipient was typed as HLA-B*35:03, thus demonstrating full UCB1 engraftment, which correlated with the data from the conventional SNP method. In our dUCBT cohort, HLA class I typing was not appropriate as a single method for assessing chimerism because there was often only one discriminating HLA class I allele between both UCB units and the patient (*Online Supplementary Table S2*). Importantly, HLA class I DNA typing combined with other techniques has already been reported in haploidentical hematopoietic stem cell transplantation to determine chimerism status.[12] More broadly, allelic KIR typing could also be a complementary tool to compare both UCB units and the recipient's genotypes. Indeed, inhibitory KIR present high allelic polymorphism, such as KIR3DL1, which also affects KIR3DL1 expression.[8] The study of allele poly-

**A**

KIR genotyping

| | 2DL1 | 2DL2 | 2DL3 | 2DL4 | 2DL5 | 3DL1 | 3DL2 | 3DL3 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 1D | 2DS5 | 3DS1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient # 11 | + | + | + | + | - | + | + | + | - | + | - | - | + | - | - |
| UCB 1 | + | - | + | + | - | + | + | + | - | - | - | - | + | - | - |
| UCB 2 | + | + | + | + | + | NULL | + | + | + | + | + | - | + | - | + |
| Recipient # 11 d+90 | + | - | + | + | - | + | + | + | - | - | - | - | + | - | - |

**B**




**C**

KIR genotyping

| | 2DL1 | 2DL2 | 2DL3 | 2DL4 | 2DL5 | 3DL1 | 3DL2 | 3DL3 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 1D | 2DS5 | 3DS1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient # 13 | + | + | + | + | A | + | + | + | + | + | + | - | + | + | + |
| UCB 1 | + | + | + | + | B | + | + | + | - | + | + | + | + | - | - |
| UCB 2 | + | + | + | + | B | + | + | + | - | + | + | + | + | - | - |
| Recipient # 13 d+90 | + | + | + | + | AB | + | + | + | + | + | + | + | + | + | + |

**D**

KIR genotyping

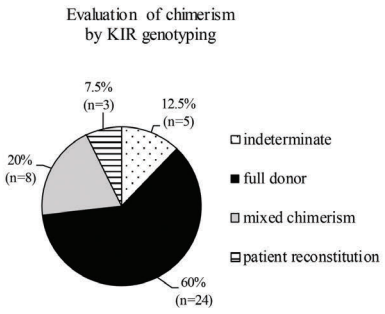
| | 2DL1 | 2DL2 | 2DL3 | 2DL4 | 2DL5 | 3DL1 | 3DL2 | 3DL3 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 1D | 2DS5 | 3DS1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient # 33 | + | + | + | + | AB | + | + | + | + | + | + | + | - | - | + |
| UCB 1 | + | + | + | + | - | + | + | + | - | + | - | - | + | - | - |
| UCB 2 | + | - | + | + | - | + | + | + | - | - | - | - | + | - | - |
| Recipient # 33 d+90 | + | + | + | + | AB | + | + | + | + | + | + | + | - | - | + |

**Figure 1.** Evaluation of hematopoietic chimerism status using KIR genotyping. (A) KIR genotyping performed for patient #11 before and after dUCBT (day 90) and on the corresponding UCB units. Genomic DNA from patients, UCB units, and recipients at different times after dUCBT were provided by the HLA laboratory (Dr A. Cesbron EFS Nantes, France) and the Hematology Department of Nantes CHU hospital (Dr L. Lodé). The presence or absence of inhibitory and activating KIR genes was determined using a KIR multiplex PCR-sequence-specific primer method as previously described.[9] For some dUCBT, KIR genotyping with an INVITROGEN kit was performed to allow the assessment of KIR2DL5 alleles and KIR3DL1 alleles were assigned as already reported.[8] KIR genes involved in the evaluation of chimerism status are highlighted in black boxes. Results indicate full donor chimerism from UCB 1. (B) Representative flow cytometry density plots leading to target KIR2DS1 using anti-KIR2DL1/S1 (anti-KIR2DL1, R&Dsystems) with anti-KIR2DL1/2/3/S2 (8C11[16]), KIR3DL1 using -KIR3DL1/S1 (Z27, Beckman Coulter) with KIR2D (EB6 + GL183, Beckman Coulter) and KIR2DL2 expression on NK cells using anti-KIR2DL2/L3/S2 (GL183, Beckman Coulter) with anti-KIR2DL3/S2 (1F12[16]) for UCB1 and UCB2 units and the recipient#11 at day 60 after dUCBT. Data were collected using a FACSCalibur (BD Biosciences), and analyzed using Flowjo 7.6.1 software (TreeStar). (C) KIR genotyping performed on patient #13 before and after dUCBT (day 90) and the corresponding UCB units. Data are indicative of mixed chimerism. (D) KIR genotyping performed on patient #33 before and after dUCBT (day 90) and the corresponding UCB units before dUCBT. Data are indicative of patient reconstitution.

**A**

| | gene | number | frequency |
|---|---|---|---|
| inhibitory KIR | 2DL5 | 28 | 70 |
| | 2DL2 | 15 | 37.5 |
| | 2DL3 | 8 | 20 |
| | 3DL1 | 4 | 10 |
| | 2DL1 | 3 | 7.5 |
| activating KIR | 2DS5 | 24 | 60 |
| | 2DS1 | 20 | 50 |
| | 3DS1 | 18 | 45 |
| | 2DS4 | 15 | 37.5 |
| | 2DS3 | 15 | 37.5 |
| | 2DS2 | 13 | 32.5 |
| | 1D | 8 | 20 |

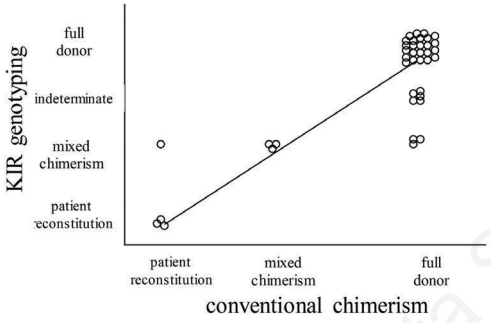
**B**



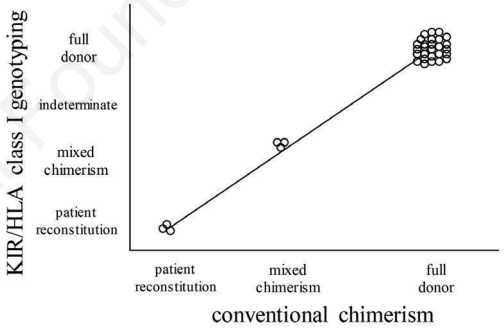

**C**

| | Evaluation of chimerism by KIR genotyping | | | | | | | | | | | | | | Evaluation of chimerism by conventional method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2DL1 | 2DL2 | 2DL4 | 2DL5 | 3DL1 | 3DL2 | 3DL3 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 1D | 2DS5 | 3DS1 | |
| patient #15 | + | + | + | A | + | + | + | + | + | + | - | + | + | + | |
| UCB 1 | + | + | + | B | + | + | + | - | + | + | + | + | - | - | |
| UCB 2 | + | + | + | B | + | + | + | - | + | + | + | + | - | - | |
| recipient #15 d+90 | + | + | + | AB | + | + | + | + | + | + | + | + | + | + | 3% UCB 1 : patient reconstitution |

**D**




**E**




**F**

| | KIR GENOTYPING | | | | | | | | | | | | | | HLA GENOTYPING | | | | | | Evaluation of chimerism by conventional method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2DL1 | 2DL2 | 2DL3 | 2DL4 | 2DL5 | 3DL1 | 3DL2 | 3DL3 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 1D | 2DS5 | 3DS1 | HLA-A* | | HLA-B* | | HLA-C* | |
| patient #39 | + | - | + | + | - | + | + | + | - | - | - | - | + | - | - | 02:01 | 11:01 | 35:01 | 41:02 | 04:01 | 17:03 | |
| UCB 1 | + | + | + | + | - | + | + | + | + | + | - | - | + | - | - | 02:01 | 11:01 | **35:03** | 14:02 | 04:01 | 08:02 | |
| UCB 2 | + | + | + | + | - | + | + | + | - | + | - | - | + | - | - | 02:01 | 11:01 | 35:01 | 14:02 | 04:01 | 08:02 | |
| recipient #39 d+90 | + | + | + | + | - | + | + | + | - | + | - | - | + | - | - | 02:01 | 11:01 | **35:03** | 14:02 | 04:01 | 08:02 | 99% UCB 1 : full donor |



**Figure 2.** KIR/HLA genotyping analysis is concordant with conventional chimerism evaluation by SNP markers. (A) Number and frequency of each inhibitory and activating KIR gene implicated in the assessment of hematopoietic chimerism using KIR genotyping. (B) Pie chart illustrating the proportions of full donor (black), mixed chimerism (gray), patient reconstitution (shaded) and indeterminate reconstitution (dotted) after evaluation of hematopoietic chimerism status using KIR genotyping in 40 recipients at day 90 after dUCBT. (C) Representative KIR genotyping performed for patient #15 before and after dUCBT (day 90) and on the corresponding UCB units illustrating discordant results between KIR genotyping analysis and conventional SNP analysis. Hematopoietic chimerism data were available for the first 3 months after dUCBT and were obtained using informative SNP markers for both patients and UCB units. Serial peripheral blood samples were separated into neutrophil and mononuclear lymphoid fractions, and provided the total white cell count. Hematopoietic chimerism after dUCBT was evaluated on whole peripheral blood cells (n=17) and/or sorted $CD3^+$ T cells (n=23). Genomic DNA from UCB units and from the recipient after-dUCBT were amplified with multiple PCR primer sets to identify markers capable of distinguishing UCB units from patient alleles. SNP analysis was performed by quantitative real-time PCR (qPCR) as described by Alizadeh *et al.*[13] on a Rotorgene Q (Qiagen). Calibration curves for each SNP marker were generated following normalization to the albumin housekeeping gene. This allowed each SNP to be quantified, and to determine the percentage from UCB units or patients. Full donor chimerism was defined as the presence of more than 95% of 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. Discriminating KIR underlined in black boxes highlight mixed chimerism based on KIR2DL5, KIR2DS1, KIR2DS4 and KIR2DS5. (D) Correlation between KIR genotyping and conventional SNP chimerism analysis from 40 dUCBT. (E) Correlation between KIR/HLA genotyping and conventional SNP chimerism analysis from 37 dUCBT on available DNA samples. (F) Representative KIR and HLA class I genotyping for patient #39, both UCB units and recipient #39 with day 90 after dUCBT. Discriminating KIR are shown by black boxes. Discriminating HLA class I alleles are shown in bold. High resolution typing for HLA-A, HLA-B and HLA-C loci was carried out using a Sequence Based Typing kit (Abbott Molecular Park, LL, USA) prospectively on all patients and UCB units before dUCBT and retrospectively on some recipients after dUCBT.

morphisms of all KIR genes by next-generation sequencing should reveal the extreme diversity of KIR genes, as recently reported,[13] and could provide the missing information needed to determine hematopoietic chimerism precisely after dUCBT, thus discriminating both UCB units and patient at KIR allele level. To evaluate KIR as chimerism markers early after dUCBT (from days 14-20), DNA samples were provided at multiple time points after dUCBT and used to evaluate the kinetics of hematopoietic chimerism for some recipients. As illustrated in Figure 3A, the absence of *KIR2DL3*, *1D*, and *KIR2DS5* genes and the presence of the *KIR2DS4* gene demonstrated UCB unit 1 reconstitution as early as day 20 after dUCBT in recipient #5. This reconstitution remained stable at days 30 and 75 after dUCBT, in accordance with the evaluation of hematopoietic chimerism by SNP-PCR established in that case only on day 75 after dUCBT (Figure 3A).

KIR genotyping enabled chimerism status to be evaluated as early as day 20 after dUCBT in ten other recipients (*data not shown*). To confirm that KIR genotyping performed early after dUCBT is linked to the reconstitution of only one UCB unit, KIR$^+$ NK cell phenotype was determined by flow cytometry from day 14 to 60 after dUCBT, as illustrated for recipient #31 (Figure 3B). Full UCB1 reconstitution was highlighted by the absence of *KIR2DL2* and *KIR2DS2* genes as illustrated in the KIR amplification patterns. The KIR$^+$ NK cell phenotype marked by the absence of KIR2DL2 on day 14 remained stable on days 28 and 60 after dUCBT. Interestingly,

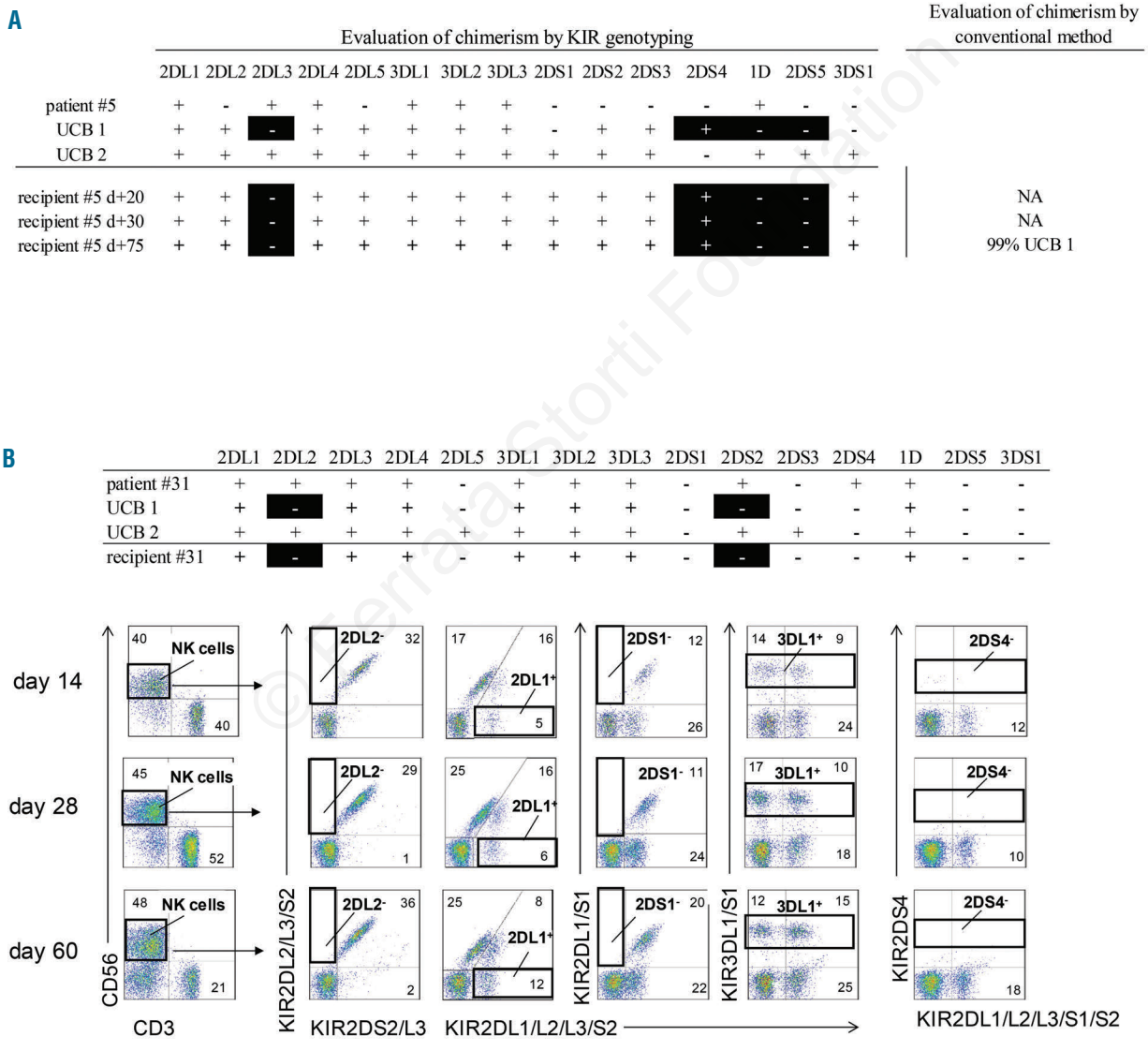



Figure 3. Early determination of chimerism status after dUCBT by KIR genotyping analysis compared to SNP markers. (A) KIR genotyping performed on patient #5 and the corresponding UCB units before dUCBT and on recipient #5 on days 20, 30 and 75 after dUCBT with conventional SNP chimerism data available on day 75 after dUCBT, illustrating the early and stable full donor engraftment. NA: Not available. (B) KIR genotyping performed for patient #31 before and after dUCBT (day 90) and on the corresponding UCB units. Representative flow cytometry density plots illustrate the early KIR$^+$ NK cell reconstitution stained with anti-KIR2DL2/L3/S2 (GL183, Beckman Coulter, Immunotech, Marseille, France), anti-KIR3DL1/S1 (Z27, Beckman Coulter), anti-KIR2DS4 (FES172, Beckman Coulter), anti-KIR2DL1/S1 (EB6, R&D systems), anti-KIR2DL3/S2 (1F12[16]), and anti-KIR2DL1/2/3/S2 (8C11[16]) for patient #31 on days 14, 28 and 60 after dUCBT. Data were collected using a FACSCalibur (BD Biosciences), and analyzed using Flowjo 7.6.1 software (TreeStar).

genotypic and phenotypic results showed that the KIR NK cell repertoire is constituted from only one UCB unit as early as day 14 after dUCBT.

Overall, our data support that KIR genes can be considered as additional markers to assess hematopoietic chimerism status as early as day 14 after dUCBT. Donor chimerism determined on bone marrow samples by semi-quantitative PCR of short tandem repeats on day 21 was predictive of subsequent neutrophil recovery.[14] However, the impact of early mixed chimerism on relapse incidence was not investigated in this study which was restricted to assessment of UCB engraftment alone. Correlation of early mixed chimerism with relapse incidence, as recently reported in allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning,[15] was not possible in our cohort of limited size since only three dUCBT with early mixed chimerism were included. However, this should be prospectively investigated in a larger dUCBT cohort to determine whether detection of mixed chimerism using KIR genotypes as early as day 14 after dUCBT could be used as an early predictive and prognostic marker of relapse.

Using KIR genotypes with supplemental HLA class I typing for some dUCBT with available DNA, we detected both engraftment with either full or mixed chimerism or graft failure after dUCBT, in concordance with the conventional SNP-quantitative PCR method. Determination of hematopoietic chimerism based on KIR genotyping can also be applied to HLA-matched unrelated and haploidentical hematopoietic stem cell transplant recipients since KIR and HLA genes are located on different chromosomes. From a clinical point of view, the determination of chimerism status after dUCBT with KIR markers as early as day +14 will allow physicians to adapt treatment therapy promptly, for example by modulating immunosuppression to manage, in particular, leukemic relapse.

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*This letter has a Supplementary Appendix.*

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## References

1. Thomson BG, Robertson KA, Gowan D, et al. Analysis of engraftment, graft-versus-host disease, and immune recovery following unrelated donor cord blood transplantation. Blood. 2000;96(8):2703-2711.
2. Elkaim E, Picard C, Galambrun C, et al. Peripheral blood cells chimerism after unrelated cord blood transplantation in children: kinetics, predictive factors and impact on post-transplant outcome. Br J Haematol. 2014; 166(4):557-565.
3. Peterlin P, Delaunay J, Guillaume T, et al. Complete donor T cell chimerism predicts lower relapse incidence after standard double umbilical cord blood reduced-intensity conditioning regimen allogeneic transplantation in adults. Biol Blood Marrow Transplant. 2015;21(1):180-184.
4. Lamba R, Abella E, Kukuruga D, et al. Mixed hematopoietic chimerism at day 90 following allogenic myeloablative stem cell transplantation is a predictor of relapse and survival. Leukemia. 2004; 18(10):1681-1686.
5. Alizadeh M, Bernard M, Danic B, et al. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. Blood. 2002; 99(12):4618-4625.
6. Koldehoff M, Steckel NK, Hlinka M, Beelen DW, Elmaagacli AH. Quantitative analysis of chimerism after allogeneic stem cell transplantation by real-time polymerase chain reaction with single nucleotide polymorphisms, standard tandem repeats, and Y-chromosome-specific sequences. Am J Hematol. 2006;81(10):735-746.
7. Triplett BM, Horwitz EM, Iyengar R, et al. Effects of activating NK cell receptor expression and NK cell reconstitution on the outcomes of unrelated donor hematopoietic cell transplantation for hematologic malignancies. Leukemia. 2009;23(7):1278-1287.
8. Gagne K, Willem C, Legrand N, et al. Both the nature of KIR3DL1 alleles and the KIR3DL1/S1 allele combination affect the KIR3DL1 NK-cell repertoire in the French population. Eur J Immunol. 2013; 43(4):1085-1098.
9. Sun JY, Gaidulis L, Miller MM, et al. Development of a multiplex PCR-SSP method for killer-cell immunoglobulin-like receptor genotyping. Tissue Antigens. 2004;64(4):462-468.
10. Hashem H, Lazarus HM. Double umbilical cord blood transplantation: relevance of persistent mixed-unit chimerism. Biol Blood Marrow Transplant. 2015;21(4):612-619.
11. Sturn A, Quackenbush J, Trajanoski Z. Genesis: cluster analysis of microarray data. Bioinforma Oxf Engl. 2002;18(1):207-208.
12. Huang X-J, Liu D-H, Liu K-Y, et al. Treatment of acute leukemia with unmanipulated HLA-mismatched/haploidentical blood and bone marrow transplantation. Biol Blood Marrow Transplant. 2009; 15(2):257-265.
13. Kidd JM, Sharpton TJ, Bobo D, et al. Exome capture from saliva produces high quality genomic and metagenomic data. BMC Genomics. 2014;15:262.
14. Avery S, Voss MH, Gonzales AM, et al. Importance of day 21 BM chimerism in sustained neutrophil engraftment following double-unit cord blood transplantation. Bone Marrow Transplant. 2012; 47(8):1056-1060.
15. Reshef R, Hexner EO, Loren AW, et al. Early donor chimerism levels predict relapse and survival after allogeneic stem-cell transplantation with reduced intensity conditioning. Biol Blood Marrow Transplant. 2014;20(11):1758-1766.
16. David G, Morvan M, Gagne K, et al. Discrimination between the main activating and inhibitory killer cell immunoglobulin-like receptor positive natural killer cell subsets using newly characterized monoclonal antibodies. Immunology. 2009;128(2):172-184.