SDC, METHODS

Phenotypic analysis of NK cells by flow cytometry

Cord blood and adult NK-cell surface phenotype was determined by four-color flow cytometry using the following mouse anti-human monoclonal antibodies (mAbs): anti-CD3-PerCP (SK7), anti-CD56-APC (B159) (BD Biosciences), anti-KIR3DL1/S1-PE (Z27), (Beckman Coulter, Immunotech, Marseille, France), anti-KIR3DL1-FITC (DX9), (Beckman Coulter, Immunotech, Marseille, France), anti-NKG2A FITC (Z199), (Beckman Coulter and EFS Nantes), anti-KIR2DL1/S1-PE (EB6), anti-KIR2DL2/3/S2-PE (GL183) (Beckman Coulter), anti-KIR2DL1/2/3/2DS1/2 (1A6), anti-KIR2DL1/2/3/S2-FITC (8C11) and anti-KIR2DS2/L3-FITC (1F12) [1]. HLA class I expression was evaluated using anti-HLA class I FITC (NaM41-1E3, EFS-PL, Nantes). Cells were also stained with a corresponding isotype-matched control mAb (BD Pharmingen, Franklin Lakes, NJ). Data was collected using a FACSCalibur (BD Biosciences) and analyzed using Flowjo 7.6.1 software (TreeStar).

Phosphoflow experiments by flow cytometry

Phosphoflow experiments were used to evaluate adult and cord blood NK cell activation. Isolated thawed PBMC were first labelled with anti-CD3-PerCP (SK7), anti-CD56-APC (B159) (BD Biosciences). After 30 minutes with PMA/ionomycin stimulation, cells were fixed with PFA 1%. Then, cells were permeabilized in a PBS 0.1% saponine solution, and phosphorylated ERK and P38 intracellular expression was measured using anti-ERK1/2-AF488 (20A) and anti-P38 MAPK-PE (36/p38 pT180/pY182) (BD Biosciences). The protocol was first validated on cord blood and adult T cells (data not shown).

CD107a mobilization assay detected by flow cytometry

Adult and cord blood NK cells were tested for their cytolytic potential with the CD107a mobilization assay after stimulation with 221, 221-Bw4 and 221-Bw6 transfected cell lines. NK cells pre-stained with CD107-PE-Cy5 (H4A3, BD Biosciences) from thawed PBMC were cultured with different target cells (221, 221-Bw4, 221-Bw6) for five hours at an E:T ratio of 1:1 with brefeldin A (Sigma) at 10 µg/mL for the last four hours. The cells were surface stained with Z27-PE, NKG2A-FITC and NKp46-allophycocyanin (9E2, BD Biosciences).

Apoptosis evaluation measured by Annexin-V staining

Two weeks stimulated cord blood cells were incubated with 221-Bw4 negative or 221-Bw4 positive targets at an E:T ratio of 1:1 for 2h at 37°C. After incubation, cells were surface stained with anti-KIR3DL1/S1-PE (Z27) (Beckman Coulter) and anti-NKp46-APC (BD Biosciences). Then, cells were resuspended in BD Biosciences Apoptosis kit Buffer and Annexin-V expression was evaluated by staining with anti-Annexin-V-PE (BD Biosciences).

Statistical analysis

Data was analyzed from the Société Francophone de Greffe de Moelle (SFGM) database with the data existing as of January 2015. Characteristics of dUCBT were described and compared. Continuous variables (expressed as medians and range) were compared between groups with Wilcoxon tests. Categorical variables (expressed as numbers and percentages) were compared between groups with χ^2 tests, Fisher's exact test or Mac Nemar test where appropriate. To analyze the likelihood of sustained UCB unit dominance, we considered variables associated with the infused UCB units (TNC cell doses, $CD34^+$ cell doses, $CD34^+$ post-thaw viability of each UCB unit) and with UCB unit/recipient matching such as sex, HLA-ABDRB1, HLA-C, HLA-Bw4, KIR ligand and KIR/KIR ligand combinations. Delay of neutrophil recovery and overall survival were estimated by the

Kaplan-Meier method. Univariate and multivariate Cox regressions were used to evaluate the impact of the KIR3DL1^{+} loser UCB unit/Bw4^{-} winner UCB unit genetic combination and other risk factors on one full UCB unit dominance and overall survival after dUCBT. Cumulative incidence was used to estimate relapse incidence and acute GvHD (death without relapse/aGvHD were considered as competing risks). Univariate and multivariate proportional hazard models of Fine and Gray were used to evaluate the impact of the KIR3DL1^{+} loser UCB unit/Bw4^{-} winner UCB unit genetic combination and other risk factors on relapse and acute GvHD incidence. Candidate variable for the multivariate models were those associated with the outcome in univariate analyses with the $p<0.20$ criterion. P-values<0.05 were considered to be statistically significant in multivariate analyses. An adjustment to the P values (q value) was performed using False Discovery Rate (FDR) correction. Analyses were performed using SAS 9.2 statistical software (SAS Institute, Inc.) and the "cmprsk" package of the R statistical software developed by Gray.

The one-way analysis of variance (ANOVA) test was used to compare HLA class I expression in adult and cord blood samples, the phosphoproteins MFI in phosphoflow experiment, the CD107a^{+} NK cell and CD107a^{+} KIR3DL1^{+} NK cell frequencies in functional assay. P-values <0.05 were considered as statistically significant.

SDC, REFERENCES

1. 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;128:172-184.

TABLE S1: Characteristics of double-umbilical cord blood transplantation with one full UCB unit dominance

PATIENTS (n=50)			
Median age at grafting (years):	53.26 [4.3-69.14]		
Patient gender			
Male	25 (50%)		
Female	25 (50%)		
Diagnosis:			
Myeloid diseases[1]	29 (58%)		
Lymphoid diseases[2]	20 (40%)		
Aplastic anemia	1 (2%)		
Disease status			
Standard risk	12 (24%)		
High risk	37 (74%)		
Not applicable	1 (2%)		
Conditioning regimen:			
Full intensity	9 (18%)		
Reduced intensity (Fluda endoxan TBI 2 grays)	41 (82%)		
TBI based	48 (96%)		
ATG			
Yes	13 (26%)		
No	37 (74%)		
GvHD prophylaxis			
CSA +/- CS	8 (16%)		
CSA + MMF	42 (84%)		
Engraftment with one full dominant UCB unit	*Winner UCB units (n=50)*		*Loser UCB units (n=50)*
Cell infused after thawing:			
Total nucleated cells x10^7/kg (median)	2.14 [1.00-7.20]		1.89 [1.18-5.26]
CD34+ cells x10^5/kg (median)	0.42 [0.10-2.70]		0.42 [0.004-3.33]
HLA compatibility patient/UCB[3]:			
6/6	1 (2%)		0
5/6	30 (60%)		31 (62%)
4/6	19 (38%)		19 (38%)
HLA compatibility UCB/UCB[3]:			
6/6		7 (14%)	
5/6		23 (46%)	
4/6		18 (36%)	
3/6		2 (4%)	

Abbreviations: TBI, total body irradiation; ATG, anti-thymoglobulin; GvHD, graft versus host disease; CSA, cyclosporine A; MMF, mycophenolate mofetil; UCB, umbilical cord blood; UCBT, umbilical cord blood transplantation; [1]include acute myeloid leukemia (AML), chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN), [2] include acute lymphoid leukemia (ALL), T-cell prolymphocytic leukemia (T-PLL), Hodgkin lymphoma, non-Hodgkin lymphoma (NHL); [3]HLA compatibility patient/UCB, and UCB/UCB at low resolution for HLA-A and HLA-B and high resolution for HLA-DRB1.

SDC, TABLE 2: Analysis of KIR3DL1^{+} loser UCB unit/Bw4^{-} winner UCB unit genetic combination influencing neutrophil recovery and relapse incidence after dUCBT with one full UCB unit dominance using Cox model

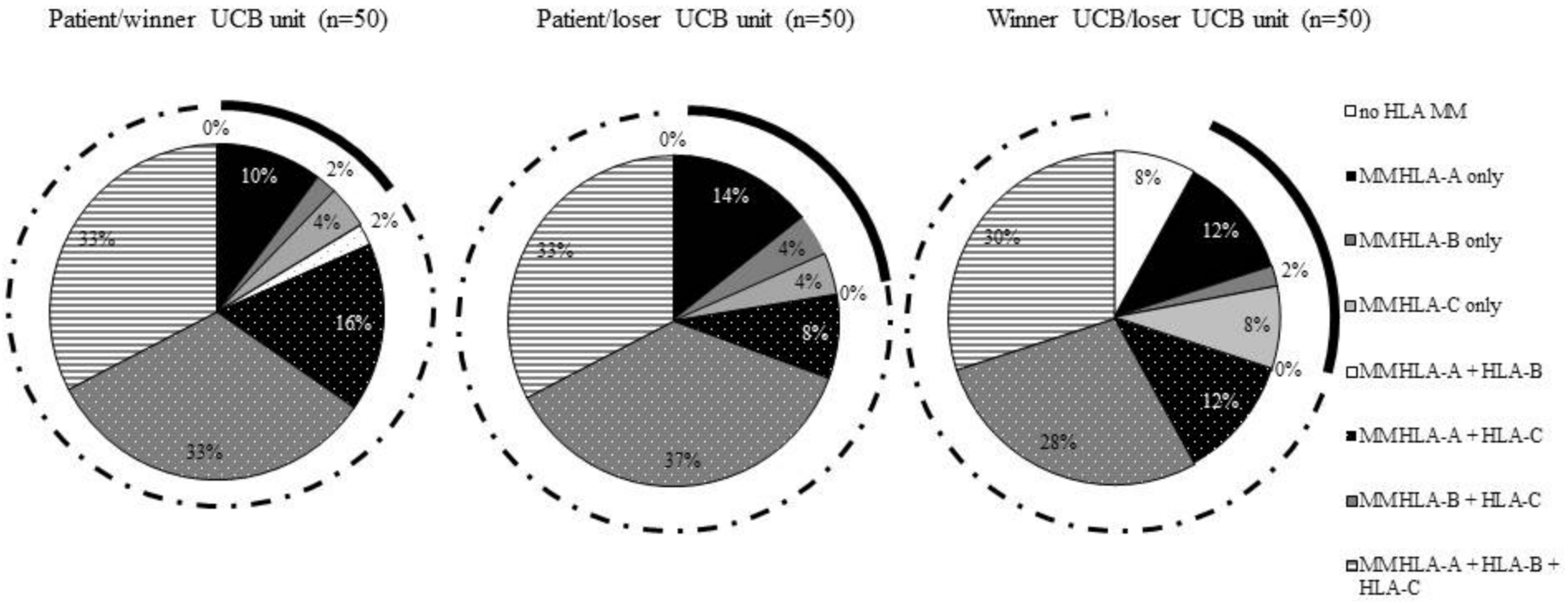
Cox multivariate analysis	Neutrophil recovery		Relapse incidence	
	HR [CI 95%]	p value	HR [CI 95%]	p value
Patients with high risk diseases (74%)	**3.08 [1.35-7.06]**	**0.008**	3.07 [0.71-13.3]	0.13
Patients with myeloid diseases (58%)	**3.23 [1.15-9.08]**	**0.027**	**5.95 [1.43-24.7]**	**0.014**
Patients having received TBI (96%)	**3.85 [1.76-8.45]**	**0.0008**	**5.78 [1.67-20.0]**	**0.0055**
Patients with no ATG (74%)	**2.91 [1.18-7.20]**	**0.021**	3.08 [0.34-27.9]	0.32
Patients with RIC regimen (82%)	**2.79 [1.22-6.37]**	**0.015**	**5.39 [1.19-24.5]**	**0.029**

Abbreviations: HR: Hazard Ratio; TBI: total body irradiation; ATG: anti-thymoglobulin; RIC: reduced intensity conditioning regimen; dUCBT: double umbilical cord blood transplantation; CI: confidence interval.

TABLE S3: Analysis of KIR3DL1^{+} loser UCB unit/Bw4^{-} winner UCB unit genetic combination influencing neutrophil recovery and relapse incidence after dUCBT with one full UCB unit dominance

Multivariate analysis	HR [CI 95%]	p value
Neutrophil recovery	**2.99[1.44-6.23]**	**0.0034**
Relapse incidence	**4.28[1.34-13.7]**	**0.0142**
Overall survival	2.16[0.77-6.07]	0.1442
Acute GvHD incidence	0.76[0.26-2.21]	0.6182

Abbreviations: HR: Hazard Ratio; dUCBT: double umbilical cord blood transplantation; CI: confidence interval; GvHD: Graft-versus-Host-Disease



SDC, FIGURE 1 : Charts representing the distribution of isolated (solid line) and cumulated (dotted line) HLA class I mismatches (MM) found between patients and winner UCB units (n=50), patients and loser UCB units (n=50) and between both UCB units (n=50) taking into account HLA-A, HLA-B and HLA-C typing at low resolution.