Natural killer cell licensing after double cord blood transplantation is driven by the self-HLA class I molecules from the dominant cord blood

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doi:10.3324/haematol.2015.138883

SUPPLEMENTAL FILE

METHODS

The present study included 20 T-cell-replete patients having undergone 2CBT at the Bone Marrow Transplantation Unit, Saint-Louis Hospital (Paris, France) between 2006 and 2012 to treat a hematological disorder (acute leukemia: n=10; aplastic anemia: n=5; non-Hodgkin lymphoma: n=3; Hodgkin disease: n=2). Fifteen patients underwent a myeloablative conditioning regimen. The median patient age was 31.2 years (range: 14-56). Patients' HLA-C, KIR genotyping and transplant characteristics are summarized in supplemental Table 1.

All had full, single cord blood chimerism at 3 months post-2CBT. Chimerism was determined using DNA extracted from whole blood or cord blood cells and PCR amplification of informative polymorphic microsatellite sequences and fragment analysis on an ABI 3130 XL sequencer (Applied Biosystems, Foster city, CA). Informativity was assessed in donor and recipient pre-2CBT blood samples.

All subjects provided their informed consent to participate in the study. The study was approved by the local institutional review board and was performed in accordance with the Declaration of Helsinki. Peripheral blood mononuclear cell (PBMCs) were isolated from freshly collected blood by density-gradient centrifugation with lymphocyte separation medium and stored in liquid nitrogen prior to further analysis.

DCB-KIR genotyping was performed with the KIR SSO genotyping test (One Lambda, Canoga Park, CA). Donors and recipients were typed with median or high resolution for HLA-A, HLA-B, HLA-C, and DRB1 using the LABType HD class I or class II locus typing test (One Lambda Canoga Park, CA).

The following anti-human monoclonal antibodies were used for functional degranulation assays of NK cell subsets: CD159a-phycoerythrin (NKG2A), CD158e1/e2-phycoerythrin (KIR3DL1/S1, Z27.3.7), CD158e/k-phycoerythrin (KIR3DL1/2, 5.133), CD158a,h-PC5.5 (KIR2DL1/S1, EB6B), CD158b1/b2-PC7 (KIR2DL2/3/S2, GL183) (Beckman Coulter, Paris France); CD3-BV510, CD56-BV421 (Biolegend, San Diego, CA), CD107a-APC.Cy7. PBMCs were thawed and incubated with APC-Cy7-CD107amAb, without cytokines, in the presence or absence of target cells (the human erythroleukemia cell line K562) at an effector ratio of 1:1 for 4 hours at 37°C in culture medium. Thereafter, cells were labeled with additional fluorescent mAbs and analyzed on a BD Biosciences Canto II flow cytometer (Becton Dickinson, Le Pont de Claix, France) running DIVA software.

Statistical analysis

The Kruskal-Wallis test was used to compare cell subsets. The threshold for statistical significance was set to $P<0.05$.

Figure S1: Gating strategy for CD56^{dim} NKG2A^{-} NK cells.

The dominant cord blood and the recipient are of HLA-C allotypes C1 and C2 recognized by KIR2DL2/L3/S2 and KIR2DL1/S1 respectively. The CD56^{dim} NKG2A^{neg}KIR^{neg} NK cells are hyporesponsive while CD56^{dim} CD94/NKG2A^{neg}KIR2DL1/S1^{pos} and KIR2DL2/L3/S2^{pos} NK cells are responsive.

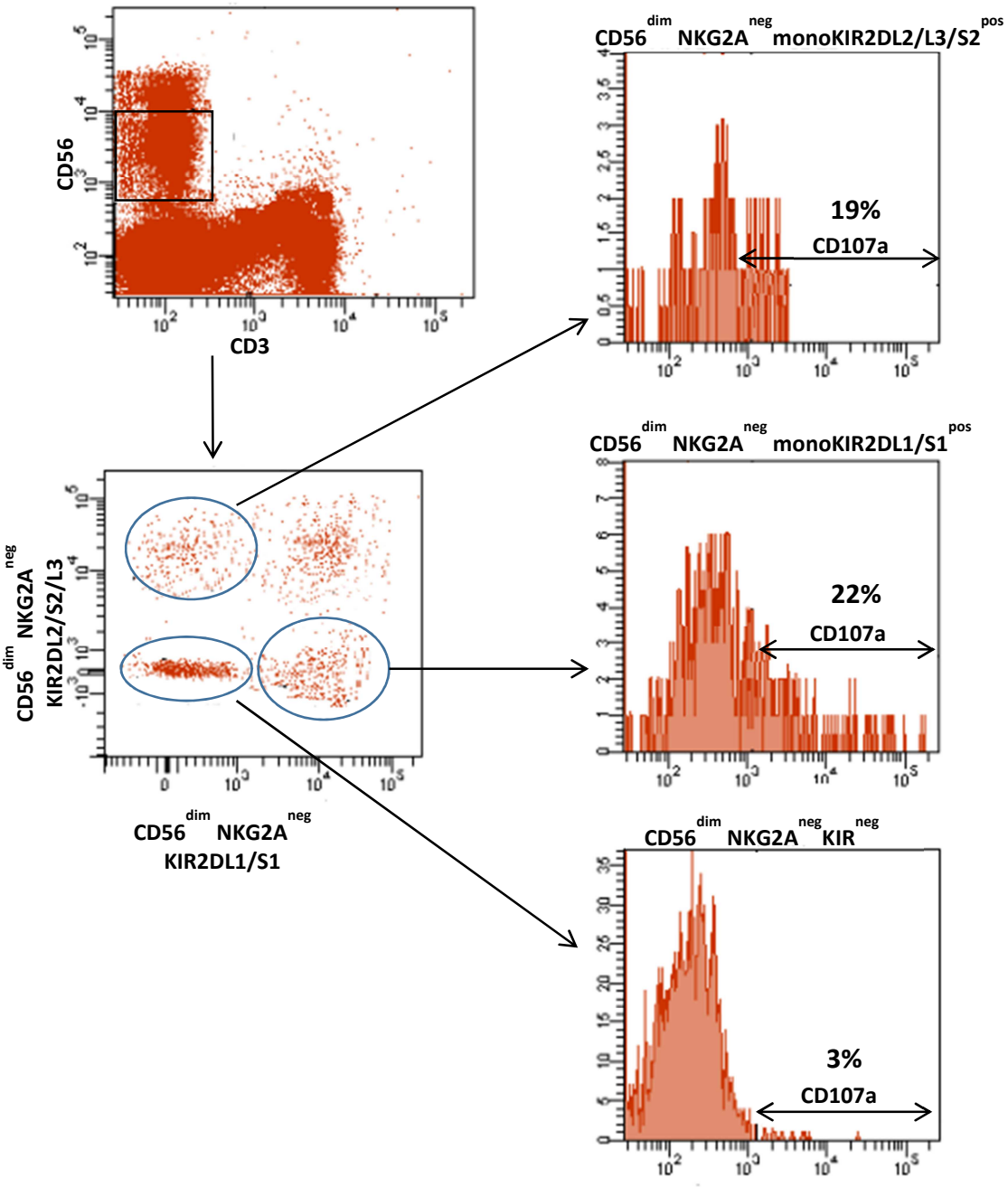

Table S1 : Study cohort definition

DCB: dominant cord blood; ndCB: non-dominant cord blood; KIR: killer immunoglobulin-like receptor; 2CBT: double umbilical cord blood stem cell transplantation;

R: recipient; *mixed chimerism at 30 to 45 days post-2CBT but full DCB chimerism at 3 months post-2CBT.

Cases	HLA-C allotypes (DCB and ndCB / R)	DCB KIR genotyping					Samples analyzed, months after 2CBT	Chimerism at 30-45 days post 2CBT
		2DL1	2DL2	2DL3	2DS1	2DS2		
	KIR-Ligand matching between DCB and R							
1	C1C2 and C1C2 / C1C2	+	-	+	+	-	M3 - M12	DCB 100%
2	C1C2 and C1C2 / C1C2	+	-	+	-	-	M6	DCB 100%
3	C1C2 and C1C2 / C1C2	+	+	+	+	+	M3 - M12	DCB 100%
4	C2C2 and C2C2 / C2C2	+	+	-	+	+	M6 – M12	DCB 100%
5	C2C2 and C2C2 / C2C2	+	-	+	+	-	M3 – M12	DCB 100%
6	C1C1 and C1C1 / C1C1	+	+	+	-	+	M3	DCB 100%
7	C1C1 and C1C1 / C1C1	+	+	+	-	+	M3	DCB 89% ndCB 11%*
8	C1C1 and C1C2 / C1C1	+	+	+	-	+	M12	DCB 100%
9	C1C1 and C1C2 / C1C1	+	-	+	-	-	M6 – M12	DCB 100%
10	C1C2 and C1C1 / C1C2	+	+	-	-	+	M3	DCB 96% - R 4%*
	KIR-ligand mismatching between DCB and R without missing KIR-Ligand in the recipient							
11	C1C1 and C1C2 / C1C2	+	+	-	+	+	M3	DCB 81% - ndCB 19%*
12	C1C1 and C1C1 / C1C2	+	+	+	-	+	M3	DCB 83% - ndCB 17%*
13	C1C1 and C2C2 / C1C2	+	+	-	-	+	M6	DCB 92% - R 8%*
14	C2C2 and C2C2 / C1C2	+	-	+	+	+	M12	DCB 72% ndCB 28%*
	KIR-ligand mismatching between DCB and R with missing KIR-Ligand in the recipient							
15	C1C2 and C1C2 / C2C2	+	+	-	+	-	M3	DCB 100%
16	C1C2 and C2C2 / C2C2	+	+	+	-	+	M6 – M12	DCB 100%
17	C1C2 and C2C2 / C2C2	+	-	+	+	+	M6	DCB 100%
18	C1C2 and C2C2 / C2C2	+	+	+	-	+	M3	DCB 71% - ndCB 29%*
19	C1C2 and C1C1 / C1C1	+	+	+	+	+	M3	DCB 90% - ndCB 10%*
20	C1C2 and C1C2 / C1C1	+	-	+	-	-	M3 - M12	DCB 100%

Table S2: NK cells count and NK subsets proportion in studied samples

NK cell type	Count cells	Subsets Proportion
$CD3^{neg}$ $CD56^{dim}$ $NKG2A^{pos}$	103 ± 50 / µl	**$CD3^{neg}$ $CD56^{dim}$ $NKG2A^{pos}$ KIR^{neg}** : 70.1% **$CD3^{neg}$ $CD56^{dim}$ $NKG2A^{pos}$ panKIR^{pos} :** 29.9% - $CD3^{neg}$ $CD56^{dim}$ $NKG2A^{pos}$ monoKIR2DL1/S1^{pos} : 32.3% - $CD3^{neg}$ $CD56^{dim}$ $NKG2A^{pos}$ monoKIR 2DL2/S2/L3^{pos} : 46.7%
$CD3^{neg}$ $CD56^{dim}$ $NKG2A^{neg}$	36 ± 33 / µl	**$CD3^{neg}$ $CD56^{dim}$ $NKG2A^{neg}$ KIR^{neg}** : 44.6% **$CD3^{neg}$ $CD56^{dim}$ $NKG2A^{neg}$ panKIR^{pos} :** 55.4% - $CD3^{neg}$ $CD56^{dim}$ $NKG2A^{neg}$ monoKIR2DL1/S1^{pos} : 29.1% - $CD3^{neg}$ $CD56^{dim}$ $NKG2A^{neg}$ monoKIR 2DL2/S2/L3^{pos} : 45.5 %