Extensive multilineage analysis in patients with mixed chimerism after allogeneic transplantation for sickle cell disease: insight into hematopoiesis and engraftment thresholds for gene therapy

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Supplementary methods.

Peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated by Ficoll gradient separation, according to a standard protocol.

Sorting of hematopoietic subpopulations

Cells were stained with specific, directly labeled monoclonal antibodies, according to the manufacturer's instructions. The following antibodies were used: FITC-labeled anti-CD3 and PE-labeled anti-CD19 (Miltenyi Biotec), PercP-Cy5.5-labeled anti-CD14 (Becton Dickinson), and Pacific Blue-labeled anti-CD15 (Beckman Coulter). An eight-color FACSCanto™ II cell analyzer and FACSAria™ II cell sorter (BD Biosciences) were used respectively for flow cytometric analysis and cell sorting, according to the manufacturer's instructions. Flow cytometry data were analyzed using FlowJo® software (TreeStar); chimerism was analyzed only when the population purity was ≥90%.

Clonogenic assay and DNA extraction

Erythroid burst-forming-units (BFU-E) and granulocyte-macrophage colony-forming-units (CFU-GM) progenitors/precursors were grown in semisolid methylcellulose medium supplemented with erythropoietin (Methocult H4435, STEMCELL Technologies Inc.) or not (Methocult H4535, STEMCELL Technologies Inc.), according to the manufacturer's instructions. Single colonies were picked out for DNA extraction.

Chimerism analysis in mature lymphoid and myeloid populations and in progenitors/precursors

Genomic DNA was extracted from whole blood or from purified cell pellets using either the manual QIAamp DNA mini kit or the automated QIAsymphony DNA mini kit (Qiagen, France), according to the manufacturer's instructions. Chimerism was first analyzed using quantitative real-time PCR assays for indel genomic polymorphisms (KimerDx kit, GenDX, Netherlands), using a method adapted from a previous publication [27]. In order to determine recipient- and donor-specific markers, samples of host and donor genomic DNA were first amplified in parallel using a panel of 30 markers in 10 multiplex PCRs (KimerDx), according to the manufacturer's instructions. Next, chimerism was quantified by quantitative real-time PCR using the chosen recipient-/donor-specific primers and probes. The percentages of

recipient or donor hematopoietic genomic DNA were calculated using the ΔΔCt method, where the threshold cycle (Ct) for the post-HSCT hematopoietic DNA was compared with the Ct for pre-HSCT host or donor DNA (set to 100%). The data were normalized against the Ct for the reference gene *RPPH1* (coding for the RNA component of RNase P ribonucleoprotein). When the recipient chimerism level exceeded 10%, an additional short tandem repeat PCR analysis was performed and then analyzed using capillary gel electrophoresis and GeneMapper software (PowerPlex 16S kit, Promega).

Mixed chimerism was defined as a recipient cell percentage above 0.05%. Increases and decreases in the level of MC were respectively defined as +2 SD or a -2 SD change from the previous result (coefficient of variation between 3 and 25%).

Donor chimerism in peripheral RBCs

The level of donor chimerism in peripheral mature RBCs was obtained by calculating the post-HSCT proportion of donor HbA, as follows: Donor RBC chimerism=[post-HSCT recipient HbA fraction (%)/donor HbA fraction (%)] x 100. For patients transplanted with AA donors, RBC donor chimerism corresponded to the percentage of HbA after HSCT. The fold change in donor chimerism in the erythroid lineage (RBCs) relative to BFU-E and CFU-GM cells was also calculated.

Supplementary Figure legends.

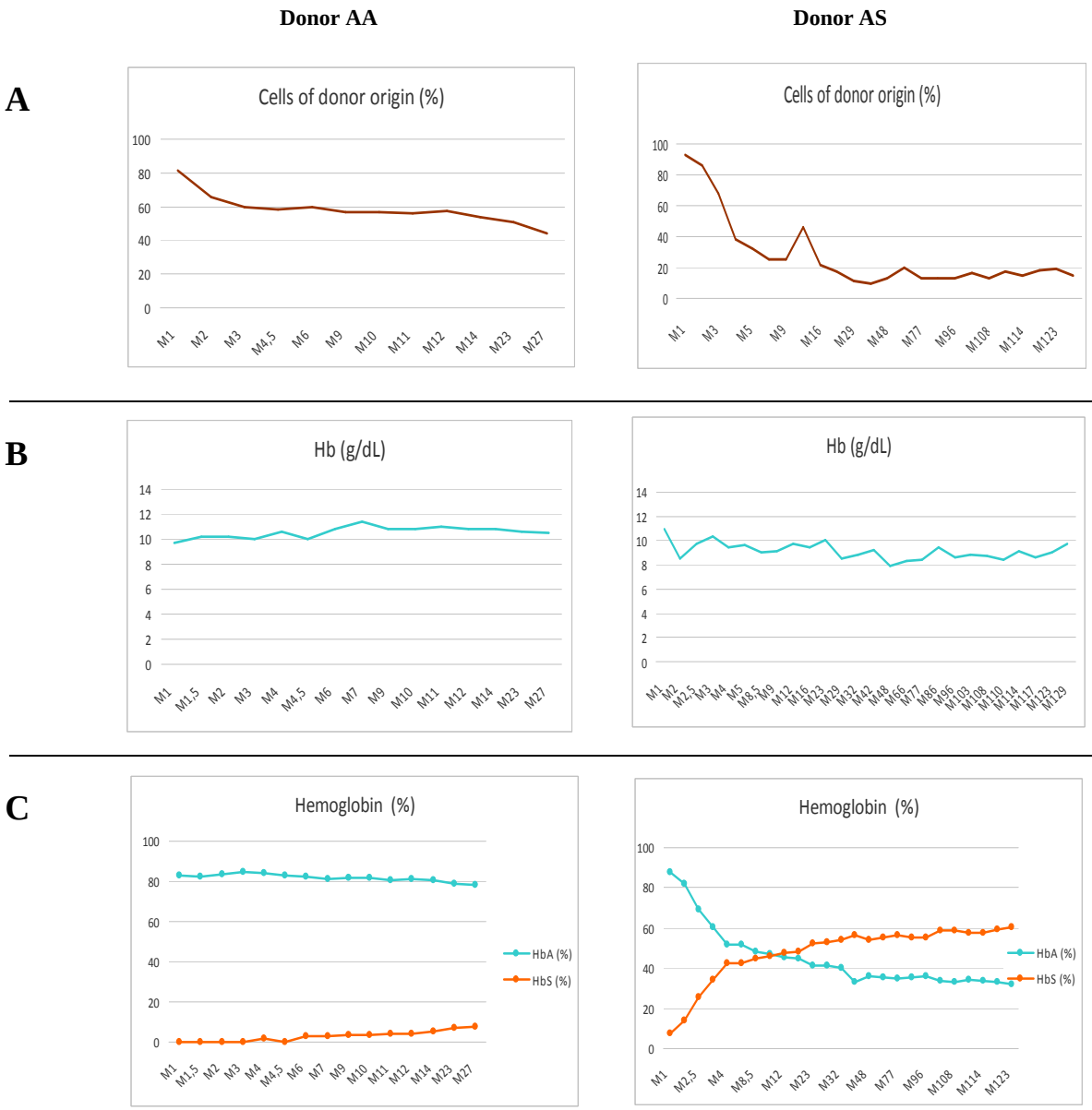
Supplementary Figure 1. Examples of time-course analyses during post-HSCT follow-up for patients with an AA donor (left) or an AS donor (right). (**A**) WBC donor chimerism; (**B**) total Hb level (g/dl); (**C**) HbA (blue line) and HbS (red line) fractions. M: months post-HSCT.

Supplementary Figure 2. Correlation between donor chimerism levels (%) in $CD15^+$ cells and in CFU-GM progenitors/precursors in patients with WBC donor chimerism <70% (group 1).

Supplementary Figure 3. Donor chimerism (%) analysis in sorted mature $CD3^+$, $CD14^+$, $CD15^+$ and $CD19^+$ cells in patients with WBC donor chimerism <70% (group 1) with either lower (**A**) or higher (**B**) donor chimerism in $CD3^+$ cells compared to the other populations.

Supplementary Figure 4. Donor chimerism (%) in $CD3^+$ and $CD15^+$ cells in patients with WBC donor chimerism < 95% (groups 1 and 2).

Supplementary Figure 1. Examples of time-course analyses during post-HSCT follow-up for patients with an AA donor (left) or an AS donor (right). (**A**) WBC donor chimerism; (**B**) total Hb level (g/dl); (**C**) HbA (blue line) and HbS (red line) fractions. M: months post-HSCT.



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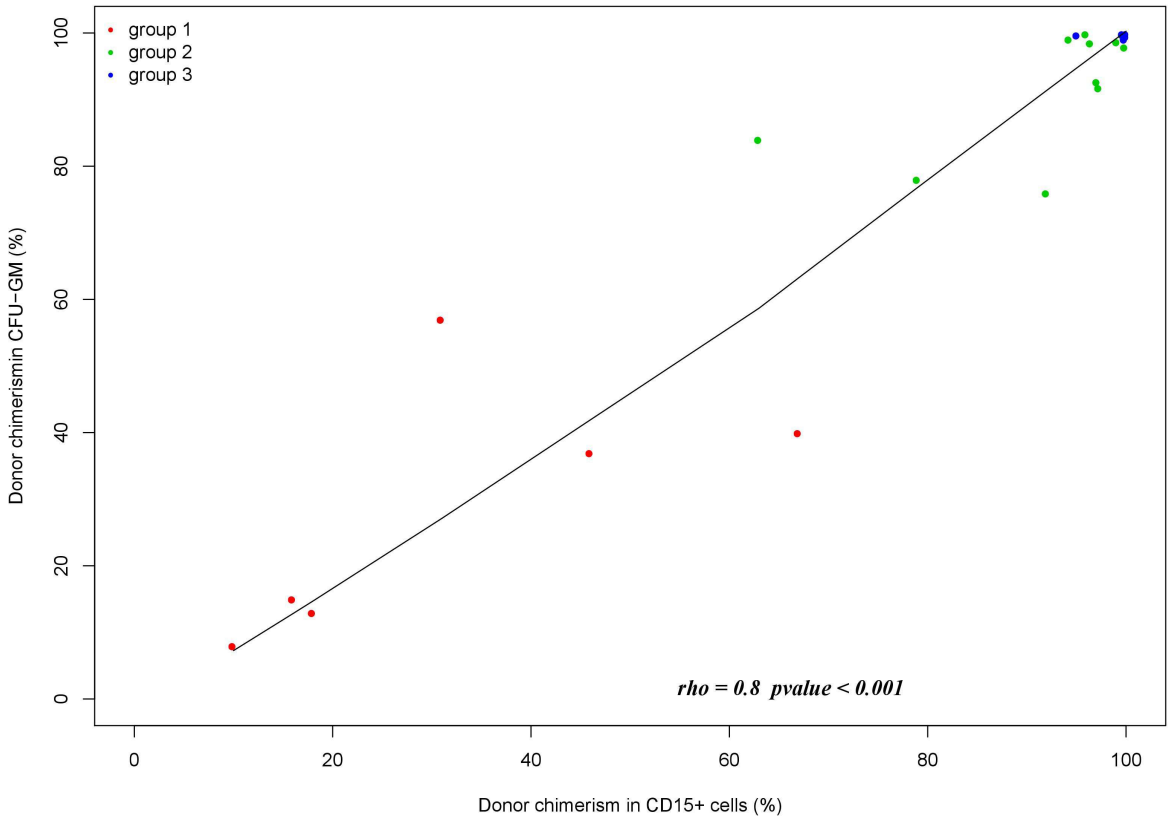

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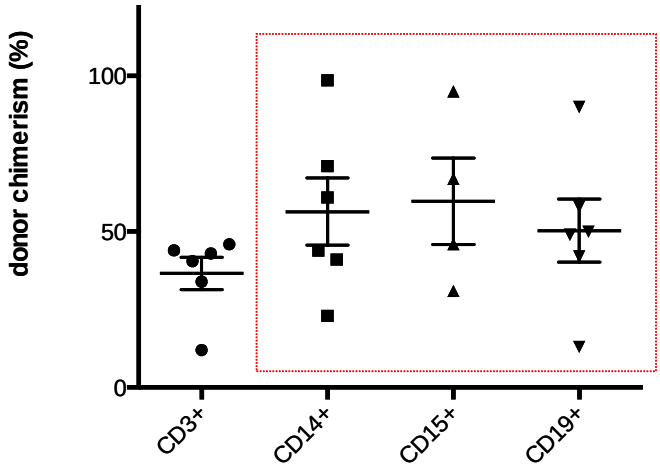
A



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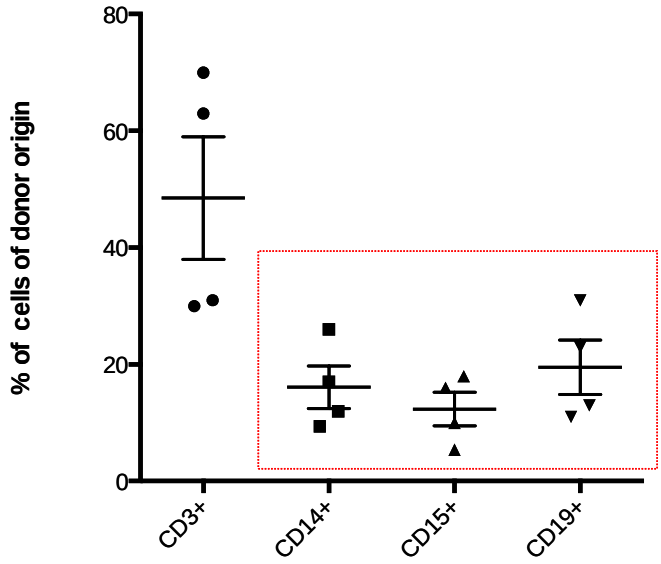

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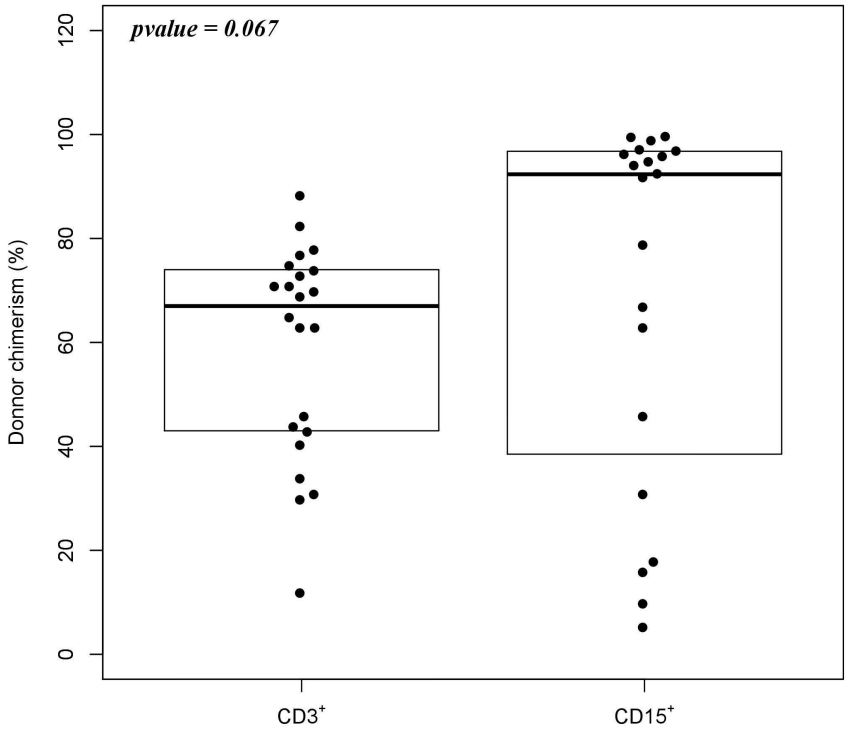

Supplementary Table 1. Characteristics of the study population and results of donor chimerism in cell subsets and progenitors/precursors

	Patient	Age at HSCT (yrs)	Follow-up after HSCT (months)	Donor Hb genotype	Donor HbS/HbA (%)	Hb level at last follow-up (g/dl)	Reticulocytes at last follow-up (G/l)	Hb fractions levels at last follow-up (%)			Donor chimerism at last follow-up (%)						
								HbA	HbS	HbF	Whole blood	CD3+	CD19+	CD14+	CD15+	BFU-E	CFU-GM
Group 1	#1	12.5	21	AA		14.2	32	84.5	0	1.1	66	40.5	90	98.5	>95	n.a.	n.a.
	#7	10.3	155	AS	38.8/57.4	13.5	31.2	50.2	37.9	0	58	34	49	71	n.a.	76	60
	#4	5.8	62	AS	33.2/54.7	10.6	40.4	54	33.3	1.4	53	44	58	44	46	39	37
	#30	8.6	55	AA		13.5	38	83.5	0	1.8	57	43	42	61	67	67	40
	#5	10.6	34	AA		10.9	140	78.3	8	3.3	44	46	50	41	n.a.	61	43
	#6	7.5	91	AA		11.8	130	79.7	5.6	1.7	19	12	13	23	31	52	57
	#2	10.8	46	AS	37.5/51.7	8.4	332	24	68.1	0	21	70	31	9.4	5.4	0	n.a.
	#8	5	133	AS	44.6/n.a.	9.7	407	31.7	60.6	1	16	31	13	12	10	17	8
	#9	3.4	153	AS	43.6/53.2	9.5	157	43.4	47.5	0	18	30	11	26	16	34	15
	#3	6.8	33	AA		10.5	227	79.8	4.3	4.4	30	63	23	17	18	31	13
Group 2	#11	4.9	54	AA		13.4	55.5	86.8	0	1	87.5	78	98.7	99.9	>99.9	98.1	97.9
	#12	8.9	58	A/β^0		9.6	161	81.2	0	3.1	91	74	98	96.3	96	98.7	>99.9
	#14	6.1	71	AS	36/49.1	12.8	16.5	48.4	38.5	1.9	91.8	73	96	n.a.	99.1	99.44	98.7
	#15	6	65	AS	35.8/52.7	13.3	21.5	50.8	38	1	91.9	88.5	96.9	n.a.	97.1	99.03	92.7
	#16	7	45	AA		13.1	36.8	86.3	0	1	81.5	69	83	93.7	92.7	n.a.	n.a.
	#17	7.5	14	AS	39.8/49.2	11.5	30	47.8	41.2	1.4	85	75	73	n.a.	92	83	76
	#18	4.1	82	A/D-Punjab		13	35.6	47.9	0	2.9	91.9	77	94.5	94.9	94.3	94.5	99.1
	#19	5.8	81	AS	32.5/54.7	13	58	53.9	32.8	1	72	71	82	n.a.	79	75	78
	#21	12.5	12	AS	37.6/47.9	12.9	55.9	44	37.8	6.5	92.4	71	97.8	>99.9	99.67	>99.9	99.76
	#25	8.3	52	AA		13.2	52	87.5	0	0	89	63	97.8	98.1	97.3	95.7	91.8
	#26	6.9	53	AS	31.5/54.8	11.6	57.8	52	33.7	3.1	76	65	90	72	63	52	84
	#28	9.2	78	AA		14.4	27	86	0	0	93.4	n.a.	n.a.	n.a.	n.a.	>99.1	99.68
	#29	6.6	38	AA		13.2	36	85.5	0	0	86.2	82.6	91.8	96.47	96.45	99.89	98.54
	#31	8.7	91	AS	35.3/52.9	14.3	27	53.7	35.3	1	89.6	n.a.	n.a.	n.a.	n.a.	96.3	n.a.
Group 3	#13	4.1	36	AS	31.5/53.3	11.7	30	53	32.4	2.2	97.7	>99.9	>99.9	n.a.	n.a.	>99.9	80
	#20	9.4	15	AS	32.5/57.3	13.7	36	55.3	33.9	1	96.3	96.4	96.7	98.2	95.1	97.2	99.7
	#22	4.6	37	AA		10.9	35.6	84	0	1.8	95.7	89	96	99.47	n.a.	99.89	98.1

#23	6.1	127	AS	38.6/57	13.4	31	49.9	39.4	0	97.4	92.8	93.5	99.72	99.8	>99.9	99.8
#24	8.3	43	AS	37.8/51.7	13.5	23	50.9	38.1	0	95.7	92.6	97.5	>99.9	n.a.	>99.9	99.15
#27	7.7	12	AA		11.5	27	79	0	5.5	99.2	94.6	98.8	>99.9	100	100	>99.9
#10	4.4	111	AS	37.5/46.9	12.5	20.6	48.8	40.3	0	99.8	99.6	99.89	99.53	99.69	100	>99.9
#32	12.1	41	AA		13.7	55	82.7	0	1.5	96.5	92.9	99	100	99.86	>99.9	99.06
#33	14.2	100	AS	34.8/52.7	12.4	24	53	33.5	1.2	99.4	97.7	99.6	>99.9	100	99.83	99.46
#34	6.4	70	AS	38.7/53.4	13.3	30	49	39.7	0	97.1	91.5	98.4	99.1	n.a.	>99.9	99.34

HSCT: Hematopoietic Stem Cell Transplantation; Hb: Hemoglobin; BFU-E: erythroid burst-forming-units; CFU-GM: granulocyte-macrophage colony-forming-units; n.a.: not available