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

Alessandra Magnani,1,2 Corinne Pondarré,3,4 Naim Bouazza,5 Jeremy Magalon,6 Annarita Miccio,7,8 Emmanuelle Six,8,9 Cécile Roudaut,1 Cécile Arnaud,3 Annie Kamdem,2 Fabien Touzot,10 Aurélie Gabrion,1 Elisa Magrin,1,2 Chloé Couzin,1 Mathieu Fusaro,11 Isabelle André,8,9 Jean-Paul Vernant,12 Eliane Gluckman,13 Françoise Bernaudin,9 Dominique Bories14* and Marina Cavazzana1,2,8,9*

1Department of Biotherapy, Necker-Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France; 2Biotherapy Clinical Investigation Center, Groupe Hospitalier Universitaire Ouest, Assistance Publique-Hôpitaux de Paris, INSERM CIC 1416, Paris, France; 3Centre de référence de drépanocytose, CHIC Centre Hospitalier Intercommunal de Créteil, Créteil, France; 4Inserm U955, Paris XII University, Créteil, France; 5Université Paris Descartes, EA7323, Sorbonne Paris Cité, CIC-1419 Inserm, Cochin-Necker, Paris, France; 6Cell Therapy Unit, Hôpital de la Conception, AP-HM, INSERM CIC BT 1409, Marseille, France; 7Laboratory of Chromatin and gene regulation during development, Imagine Institute, Paris, France; 8Paris Descartes–Sorbonne Paris Cité University, Imagine Institute, Paris; 9Human Lymphohematopoiesis Laboratory, Inserm UMR 1163, Imagine Institute, University Paris Descartes Sorbonne Paris Cité, Paris, France; 10Department of Immunology-Allergy-Rheumatology, CHU Sainte-Justine, University of Montreal, Montreal, Quebec, Canada; 11Study Center for Primary Immunodeficiencies, Assistance Publique-Hôpitaux de Paris (AP-HP), Necker-Enfants Malades University Hospital, Paris, France; 12Hematology Department, Pitié-Salpêtrière Hospital, Paris, France; 13Monacord Hôpital Saint Louis Paris, Centre Scientifique de Monaco, Monaco and Eurocord, Hôpital Saint Louis, Université Paris Diderot, Paris, France and 14Hématologie Moléculaire, Hôpital Henri Mondor, Université Paris Est, Créteil, France

*These authors are co-senior authors

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Correspondence: ALESSANDRA MAGNANI - alessandra.magnani@aphp.fr
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.
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 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 levels at last follow-up (%) | Donor chimerism at last follow-up (%) |
|---------|-------------------|------------------------------|-------------------|-------------------|-----------------------------------|--------------------------------------|-----------------------------------|-------------------------------------|
|         |                   |                              |                   |                   |                                   |                                      |                                   |                                      |
| Group 1 |                   |                              |                   |                   |                                   |                                      |                                   |                                      |
| #1      | 12.5              | 21                           | AA                | 38.8/57.4         | 14.2                              | 32                                   | 84.5                             | 0                                  |
| #7      | 10.3              | 155                          | AS                | 33.2/54.7         | 10.6                              | 40.4                                 | 54                               | 33.3                               |
| #30     | 8.6               | 55                           | AA                | 13.5              | 38                                | 83.5                                 | 57                               | 43                                 |
| #5      | 10.6              | 34                           | AA                | 10.9              | 140                               | 78.3                                 | 44                               | 46                                 |
| #6      | 7.5               | 91                           | AA                | 11.8              | 130                               | 79.7                                 | 19                               | 12                                 |
| #2      | 10.8              | 46                           | AS                | 37.5/51.7         | 8.4                               | 332                                 | 24                               | 68.1                               |
| #8      | 5                 | 133                          | AS                | 44.6/n.a.         | 9.7                               | 407                                 | 31.7                             | 60.6                               |
| #9      | 3.4               | 153                          | AA                | 43.6/53.2         | 9.5                               | 157                                 | 43.4                             | 47.5                               |
| #3      | 6.8               | 33                           | AA                | 10.5              | 227                               | 79.8                                 | 30                               | 63                                 |
| Group 2 |                   |                              |                   |                   |                                   |                                      |                                   |                                      |
| #11     | 4.9               | 54                           | AA                | 13.4              | 55.5                              | 86.8                                 | 87.5                             | 78                                 |
| #12     | 8.9               | 58                           | A/β^0             | 9.6               | 161                               | 81.2                                 | 91                               | 74                                 |
| #14     | 6.1               | 71                           | AS                | 36/49.1           | 12.8                              | 16.5                                 | 48.4                             | 38.5                               |
| #15     | 6                 | 65                           | AS                | 35.8/52.7         | 13.3                              | 21.5                                 | 50.8                             | 38.1                               |
| #16     | 7                 | 45                           | AA                | 13.1              | 36.8                              | 86.3                                 | 81.5                             | 69                                 |
| #17     | 7.5               | 14                           | AS                | 39/49.2           | 11.5                              | 30                                   | 47.8                             | 41.2                               |
| #18     | 4.1               | 82                           | A/D-Punjab        | 13                | 35.6                              | 47.9                                 | 91.9                             | 77                                 |
| #19     | 5.8               | 81                           | AS                | 32.5/54.7         | 13                               | 58                                   | 53.9                             | 32.8                               |
| #21     | 12.5              | 12                           | AS                | 37.6/47.9         | 12.9                              | 55.9                                 | 44                               | 37.8                               |
| #25     | 8.3               | 52                           | AA                | 13.2              | 52                                | 87.5                                 | 89                               | 63                                 |
| #26     | 6.9               | 53                           | AS                | 31.5/54.8         | 11.6                              | 57.8                                 | 52                               | 33.7                               |
| #28     | 9.2               | 78                           | AA                | 14.4              | 27                                | 86                                   | 93.4                             | n.a.                               |
| #29     | 6.6               | 38                           | AA                | 13.2              | 36                                | 85.5                                 | 86.2                             | 82.6                               |
| #31     | 8.7               | 91                           | AS                | 35.3/52.9         | 14.3                              | 27                                   | 53.7                             | 35.3                               |
| Group 3 |                   |                              |                   |                   |                                   |                                      |                                   |                                      |
| #13     | 4.1               | 36                           | AS                | 31.5/53.3         | 11.7                              | 30                                   | 53                               | 32.4                               |
| #20     | 9.4               | 15                           | AS                | 32.5/57.3         | 13.7                              | 36                                   | 55.3                             | 33.9                               |
| #22     | 4.6               | 37                           | AA                | 10.9              | 35.6                              | 84                                   | 95.7                             | 89                                 |

**Whole blood CD3+ CD19+ CD14+ CD15+ BFU-E CFU-GM**

- **HbA**
- **HbS**
- **HbF**
- **Whole blood CD3+ CD19+ CD14+ CD15+ BFU-E CFU-GM**
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #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