Analysis of RBC properties in patients with SCD treated with LentiGlobin gene therapy

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Introduction

Sickle cell disease (SCD) results from a homozygous missense mutation in the β-globin gene that causes polymerization of hemoglobin S. Therefore, gene therapy is a highly promising therapeutic strategy in SCD. The Phase 1/2 HGB-205 (NCT02151526) clinical study in France is evaluating the safety and efficacy of LentiGlobin gene therapy, which consists of autologous CD34+ cells transduced with a lentiviral vector encoding a human β-globin gene with a point mutation (T87Q) that confers anti-sickling properties. Data from the first successfully treated patient have been published (Ribeil et al., 2017 NEJM 376, 848-855).

RBCs of SCD patients are known to present physical, biochemical and functional abnormalities. They are more rigid, due to the increased density related to dehydration which promotes the HbS polymerization. They have increased adhesion properties and they present an impaired oxygen transport. Moreover, a high intra vascular hemolysis level and % of dense red blood cells (DRBC) have been shown to be strongly correlated to chronic complications (Kato et al., 2006 Blood 107, 2279–2285.; Bartolucci et al., 2012 Blood 120, 3136–3141.).

Presently, three patients (1204, 1207 and 1208) have been treated in HGB-205 study in France. In order to establish the effect of β^{AT87Q}-globin production on red blood cell properties, we have analyzed membrane properties, hemolysis markers, morphology, hemoglobin content, and the extent of HbS polymerization.

Methods

Whole blood samples were obtained from the 3 patients with SCD (1204, 1207 and 1208) treated in HGB-205 during their clinical follow-up.

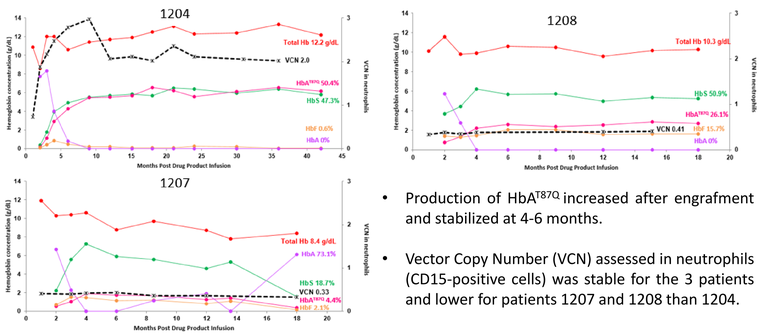
All experiments were performed within 36h after blood draw except for HbF distribution which was performed after thawing of RBCs frozen in glycerol within the same 36h and kept at -80°C. Results obtained from patients during their follow-up were compared against untreated $\beta^S\beta^S$ patients (SS) (n=12 for O_2 dissociation and association curves, n=20 for deformability assay, n=11 for adhesion assay) and healthy donors (HD) (n=12 for O_2 dissociation and association curves, n=4 for density curve, n=10 for deformability assay, n=3 for adhesion assay).

Note: Due to chronic transfusion, some of the data from patient 1207 is uninterpretable and are not presented.

Clinical Status Post drug product (DP) Infusion

Patient	Genotype	Last visit			Acute chest syndrome (ACS) and vaso-occlusive crisis (VOCs) following Lentiglobin DP infusion
		Time	Total Hb	%HbA^{T87Q}	
1204	βS/βS	Month 42	12.2 g/dL	50.4%	1 VOC at M30 (following an episode of acute gastroenteritis)
1207	βS/βS	Month 18	8.4 g/dL	4.4%	Patient has been on chronic transfusions and hydroxyurea treatment after 2 episodes of ACS at approximately 6 and 8 months after DP infusion; 1 VOC occurred since patient has been on hydroxyurea and chronic transfusions.
1208	βS/β0	Month 18	10.3 g/dL	26.1%	no episodes of VOCs or ACS

Hemoglobin and VCN follow-up since LentiGlobin gene therapy

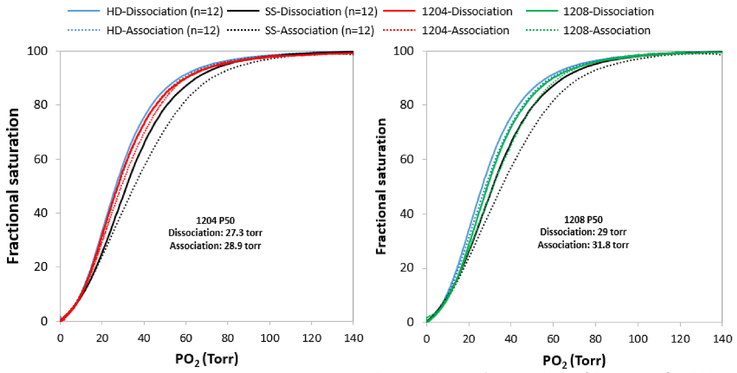


- Production of HbA^{T87Q} increased after engraftment and stabilized at 4-6 months.
- Vector Copy Number (VCN) assessed in neutrophils (CD15-positive cells) was stable for the 3 patients and lower for patients 1207 and 1208 than 1204.

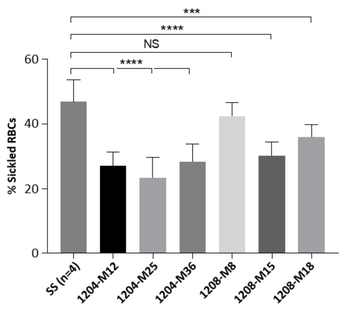
Conflict of interest disclosures:
N. Hebert: there are no relationships to disclose. **E. Magrin:** there are no relationships to disclose. **A. Miccio:** there are no relationships to disclose. **L. Kiger:** there are no relationships to disclose. **K.-A. Nguyen-Peyre:** there are no relationships to disclose. **L. Joseph:** there are no relationships to disclose. **M. Semeraro:** there are no relationships to disclose. **A. Magnani:** there are no relationships to disclose. **C. Couzin:** there are no relationships to disclose. **W. El Nemer:** *Imara*: Research Funding. **F. Pirenne:** there are no relationships to disclose. **O. Negre:** *Bluebird Bio*: Employment, Equity Ownership, Other: Salary. **J. A. Ribeil:** *Bluebird Bio*, inc.: Employment, Equity Ownership. **P. Leboulch:** *bluebird bio*: Consultancy, Equity Ownership, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties. **I. André-Schmutz:** there are no relationships to disclose. **P. Bartolucci:** *Addmedica*: Research Funding; *GBT*: Membership on an entity's Board of Directors or advisory committees; *Fondation Fabre*: Research Funding; *Novartis US*: Membership on an entity's Board of Directors or advisory committees. **M. Cavazzana:** there are no relationships to disclose.

Extent of HbS polymerization

HbS polymerization level was assessed by O_2 dissociation and association curves and by measuring the level of sickling by microscopic observation after RBCs were incubated 20 minutes under 10% O_2.

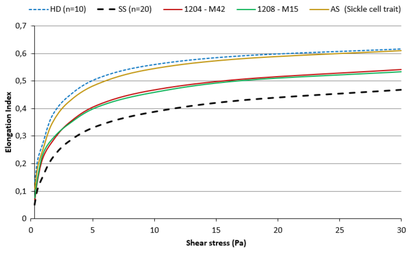


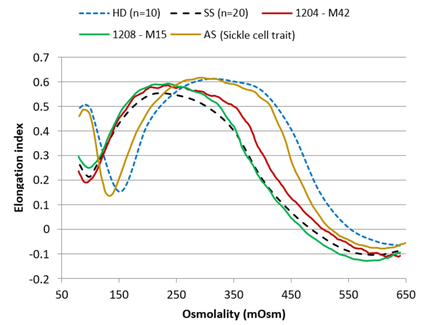
- Dissociation and association of O_2 curves for RBCs isolated from 1204 and 1208 and performed 36 and 8 months post infusion, respectively showed only a slight increase in P50 during re-oxygenation, indicating anti-sickling capability of transgenic HbA^{T87Q} and low levels of HbS polymerization.
- Significant reduction in percentage of sickled RBCs was observed for patient 1204 and patient 1208 starting at M12 and M15 post LentiGlobin gene therapy, respectively, compared to untreated patient with SCD. RBCs of patients 1204 have better anti-sickling performance than those of patient 1208. (Mann-Whitney test (****: P<0.0001 - ***: P<0.0002 – ns: not statistically significant).



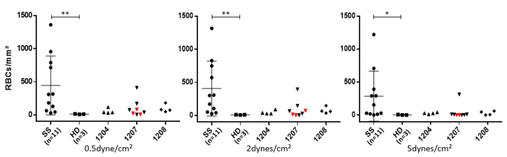
Membrane properties

Membrane deformability of RBCs was assessed under increasing Shear force (from 0.3 to 30 Pa) and in osmolality gradient using the Lorrca (Laser Optical Rotational Cell Analyzer). Elongation index was calculated as the ratio of the length (A) and width (B) of a cell, where (A–B) was divided by (A+B). RBCs adherence to surfaces coated with thrombospondin or fibronectin were performed under 0.5 dyne/cm² and assessed by microscopic observation under increasing shear stress (at 0.5, 2 and 5 dynes/cm²). Different time points are shown for the HGB-205 patients. Red points correspond to crisis day (ACS or VOC).

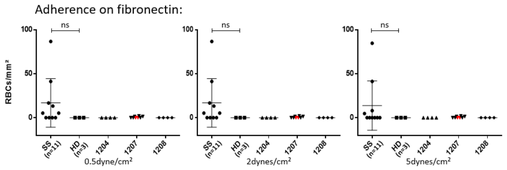




Adherence on thrombospondin



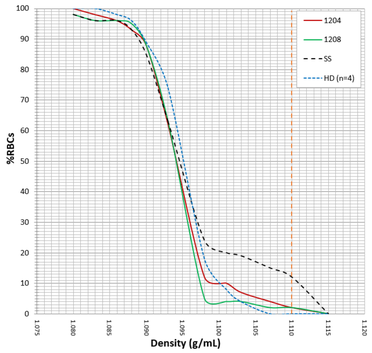
Adherence on fibronectin:



- Results of deformability showed an intermediate state of deformability for patients 1204 and 1208 as compared to HD, AS and patients with SCD.
- The shift on the left observed under osmotic gradient correspond to a reduced overall hydration with both a lower membran flexibility and a upper intracellular viscosity as compared to HD.
- Adherence profile for RBCs from patients 1204 and 1208 was more similar to HD than to untreated patients with SCD. Mann-Whitney test (**: P<0.01 - *: P<0.05 – ns: not statistically significant).

RBCs density

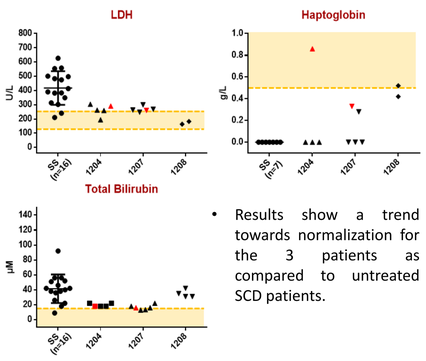
Density was assessed with the phthalate density-distribution technique in separate glass capillary hematocrit tubes, ranging from 1.080 to 1.115 g/mL. Percentage of DRBCs was calculated as the percentage of RBCs with d > 1.110 g/mL. Orange vertical dotted line shows the cut-off for dRBCs.



- Patients 1204 (at 42 months) and 1208 (at 15 months) had 2% dense RBCs (ranging from 0% to 4% since DP infusion) compared to 0% for healthy donors (n=4) and 12% for the untreated SS patient (one representative patient).
- The shift toward the left observed for patients 1204 and 1208 is due to their associated thalassemia (αα/α- and βS/β0, respectively).

Hemolysis markers

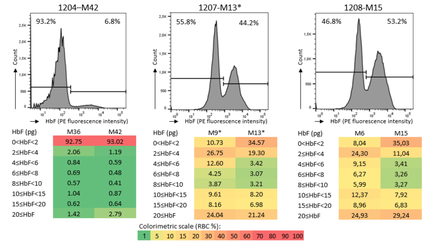
Hemolysis markers for patients 1204, 1207 and 1208 were compared to SS. Different time points are shown for the HGB-205 patients. Red points correspond to crisis day (ACS or VOC) and orange areas correspond to normal values.



- Results show a trend towards normalization for the 3 patients as compared to untreated SCD patients.

HbF quantification and distribution

Distribution of HbF levels within RBCs was assessed by flow cytometry using anti-HbF antibody coupled to phycoerythrin (PE) (patent Wo2018083426). Quantification of HbF content [pg] in RBCs was performed using normalization of anti-HbF-PE fluorescence intensity with beads of standardized florescence.



HbF (pg)	M36	M42
0<HbF<2	92.75	93.02
2≤HbF<4	2.06	1.19
4≤HbF<6	0.84	0.59
6≤HbF<8	0.69	0.48
8≤HbF<10	0.57	0.41
10≤HbF<15	1.04	0.87
15≤HbF<20	0.62	0.64
20≤HbF	1.42	2.79

HbF (pg)	M9*	M13*
0<HbF<2	10.73	34.57
2≤HbF<4	26.75	19.30
4≤HbF<6	12.60	3.42
6≤HbF<8	4.25	3.07
8≤HbF<10	3.87	3.21
10≤HbF<15	9.61	8.20
15≤HbF<20	8.16	6.98
20≤HbF	24.04	21.24

HbF (pg)	M6	M15
0<HbF<2	8,04	35,03
2≤HbF<4	24,30	11,04
4≤HbF<6	9,15	3,41
6≤HbF<8	6,27	3,26
8≤HbF<10	5,99	3,27
10≤HbF<15	12,27	7,92
15≤HbF<20	8,96	6,83
20≤HbF	24,93	29,24

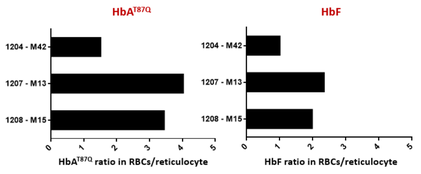
Colorimetric scale (RBC %):
1 5 10 15 20 30 40 50 60 70 80 90 100

- HbF distribution histograms for patients 1207 and 1208 revealed two populations, while most RBCs for patient 1204 had low HbF levels.

*Patient 1207 HbA transfused level was 9.9% and 5.3% at month 9 and 13, respectively.

Hemoglobin ratios in RBCs/reticulocytes

Hemoglobin content in total RBCs and reticulocytes (CD71-positive cells sorted) was assessed by reverse-phase HPLC and expressed as the ratio in RBCs/reticulocytes.



- Both HbA^{T87Q} and HbF were enriched in total RBCs compared to reticulocytes, indicating an improved survival of RBCs due to higher expression of anti-sickling hemoglobin. Greater enrichment in RBCs versus reticulocytes was observed for HbA^{T87Q} than for HbF.

*Patient 1207 HbA transfused level was 5.3% at month 13.

Conclusions

Our results suggest an improvement in RBC properties for 2 (1204 and 1208) of the 3 patients with SCD treated with LentiGlobin gene therapy in the HGB-205 clinical trial, given:

- Decrease in HbS polymerization showed by dissociation/association of O_2 curves and *in vitro* RBC sickling assays
- RBC density distribution comparable to HD
- Improvement in membrane deformability and surface adherence; however, overall membrane flexibility and internal viscosity remain more similar to untreated patients with SCD
- Trend towards normalization of hemolysis markers, consistent HbA^{T87Q} production (6.1 g/dL for 1204 and 2.7 g/dL for 1208 at last visit) and stable total Hb levels (12.2 g/dL for 1204 and 10.3 g/dL for 1208 at last visit)
- Increase in HbA^{T87Q} and HbF content in RBCs versus reticulocytes, which suggests an improved survival of RBC populations expressing anti-sickling Hb.