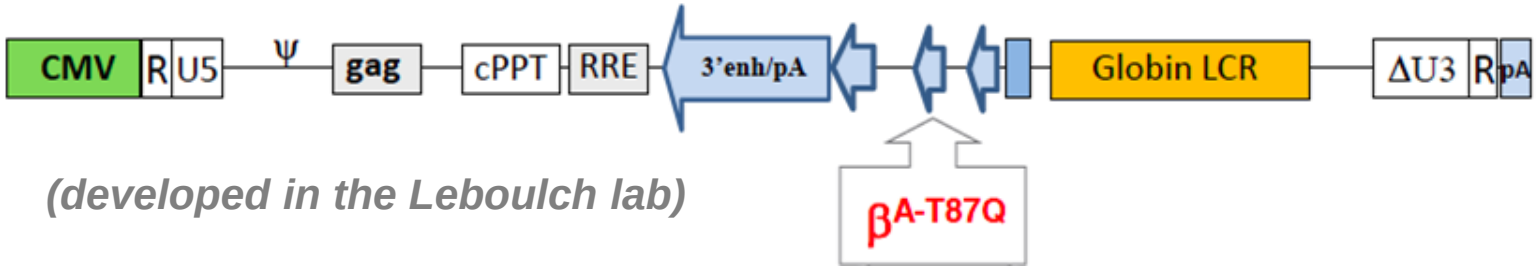
Abstract 202

Outcomes of Gene Therapy for Severe Sickle Disease and Beta-Thalassemia Major Via Transplantation of Autologous Hematopoietic Stem Cells Transduced *Ex Vivo* with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector

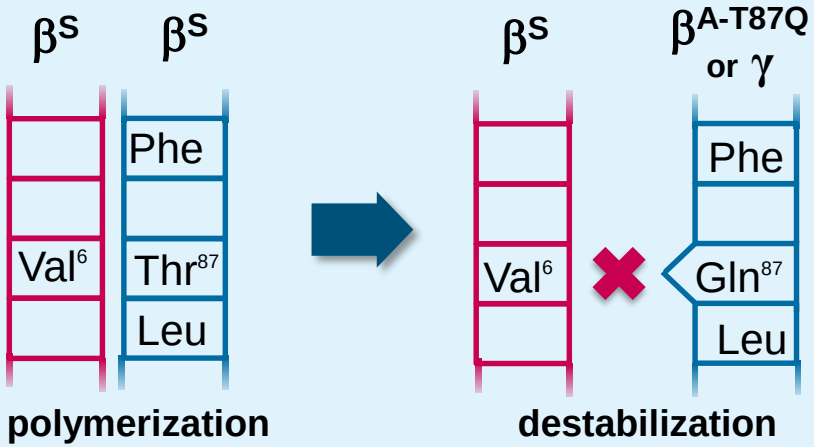
Marina Cavazzana, Jean-Antoine Ribeil, Emmanuel Payen, Felipe Suarez, Yves Beuzard, Fabien Touzot, Resy Cavallesco, Francois Lefrere, Stany Chretien, Philippe Bourget, Fabrice Monpoux, Corinne Pondarre, Benedicte Neven, Manfred Schmidt, Christof von Kalle, Frans A. Kuypers, Laura Sandler, Sandeep Soni, Olivier Hermine, Stephane Blanche, Mariane De Montalembert, Salima Hacein-Bey-Abina, and Philippe Leboulch

LentiGlobin BB305: Rationale for use in sickle cell disease



(developed in the Leboulch lab)

- $\beta^{A\text{-}T87Q}$-globin incorporates an anti-sickling amino acid substitution also found in γ globin[1]
- $\beta^{A\text{-}T87Q}$-globin and γ globin inhibit HbS polymerization[2]



Data suggest that ≥ 30% anti-sickling hemoglobin (Hb^{AT87Q} + HbF) could provide disease-modifying clinical benefit[3]

1. Takekoshi & Leboulch, PNAS 1995; 2. Ngo et al., Br.J.Haematol 2012; *Pawliuk et al, Science 2001; 3. Andreani et al Haematologica 2011*

HGB-205: Phase 1/2, single center, open-label, safety and efficacy study

- Study conducted at Hôpital **Necker Enfant Malades** / Imagine Institut des Maladies Génétiques, Paris, France
- 5 subjects treated: 1 with severe sickle cell disease, 4 with beta-thalassemia major
  as of November 10, 2015

Severe Sickle Cell Disease

Key Enrollment Criteria

- Ages 5 to 35
- Poor prognostic risk factors
- Inadequate response to hydroxyurea

Subject 1204 Pre-Study Status

male, age 13, β^S/β^S

Prior Treatments	• 7 years of hydroxyurea with continued VOCs • Monthly transfusion program since 2010 with improvement in VOCs
Clinical Status	• 1-3 VOCs per year on hydroxyurea • ACS x 2 (last in 2010) • Bilateral hip osteonecrosis • Splenectomy

Severe SCD Subject 1204: Cellular product characteristics and treatment-related safety

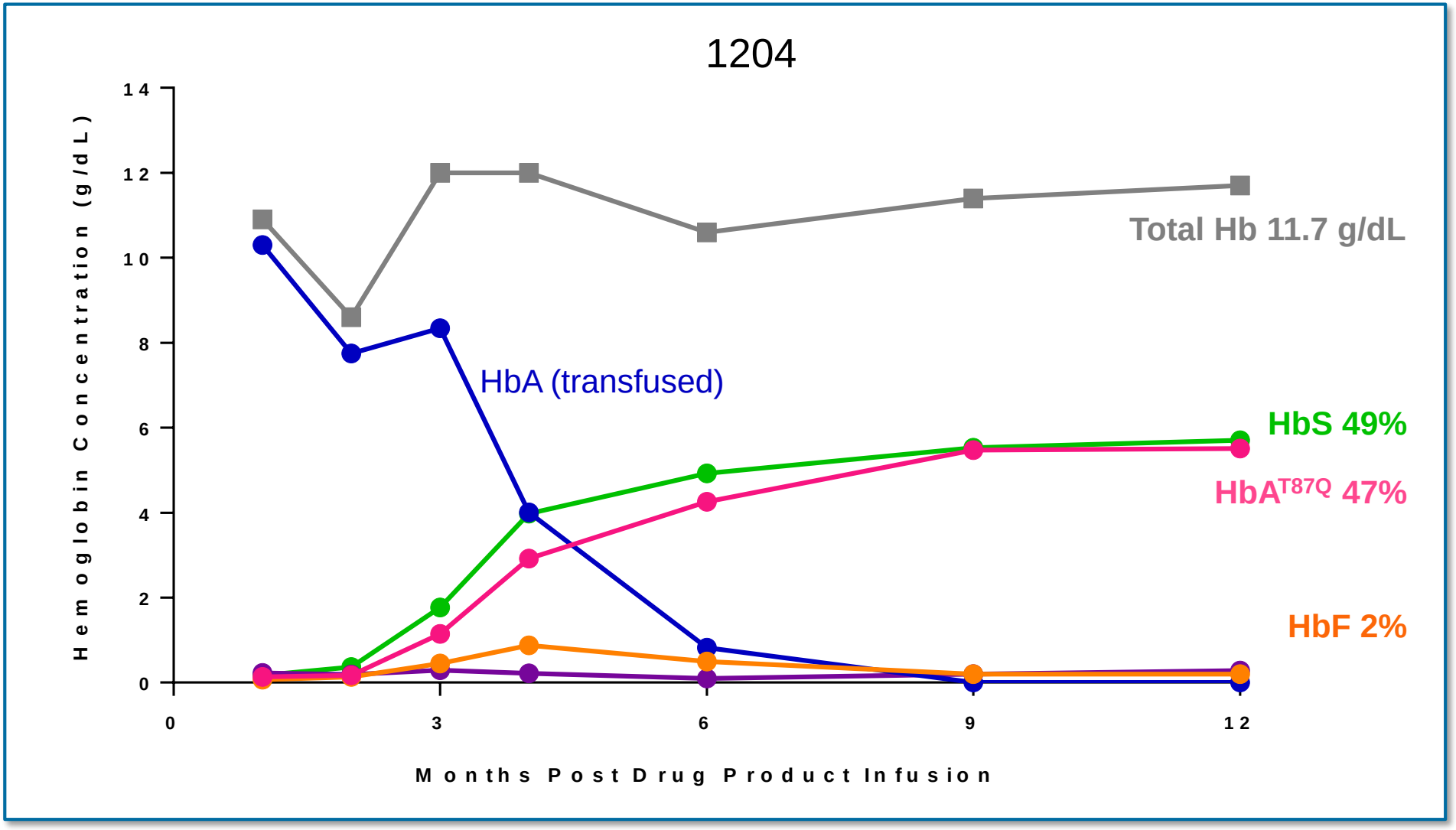
	Subject 1204
CD34+ Cell Dose (x10^6/kg)	5.6
VCN in Drug Product[1]	1.0, 1.2
Follow-up	13 months
Neutrophil engraftment[2]	Day + 37
Platelet engraftment[3]	Day + 91
Non-laboratory ≥Grade 3 AEs	None
SAEs post-infusion	None

- **Polyclonal reconstitution (2,076 unique integration events at 6 months*) without clonal dominance or detection of replication competent lentivirus**

1. VCN: number of vector copies per diploid genome; 2. ANC ≥ 500 for 3 consecutive days; 3. Unsupported platelet count ≥ 50,000/µL.

Data as of November 10, 2015 *12m insertional site analysis still in progress

Severe SCD Subject 1204**:** Equal ratio of anti-sickling hemoglobin (HbA^{T87Q} + HbF) and HbS by 9 months

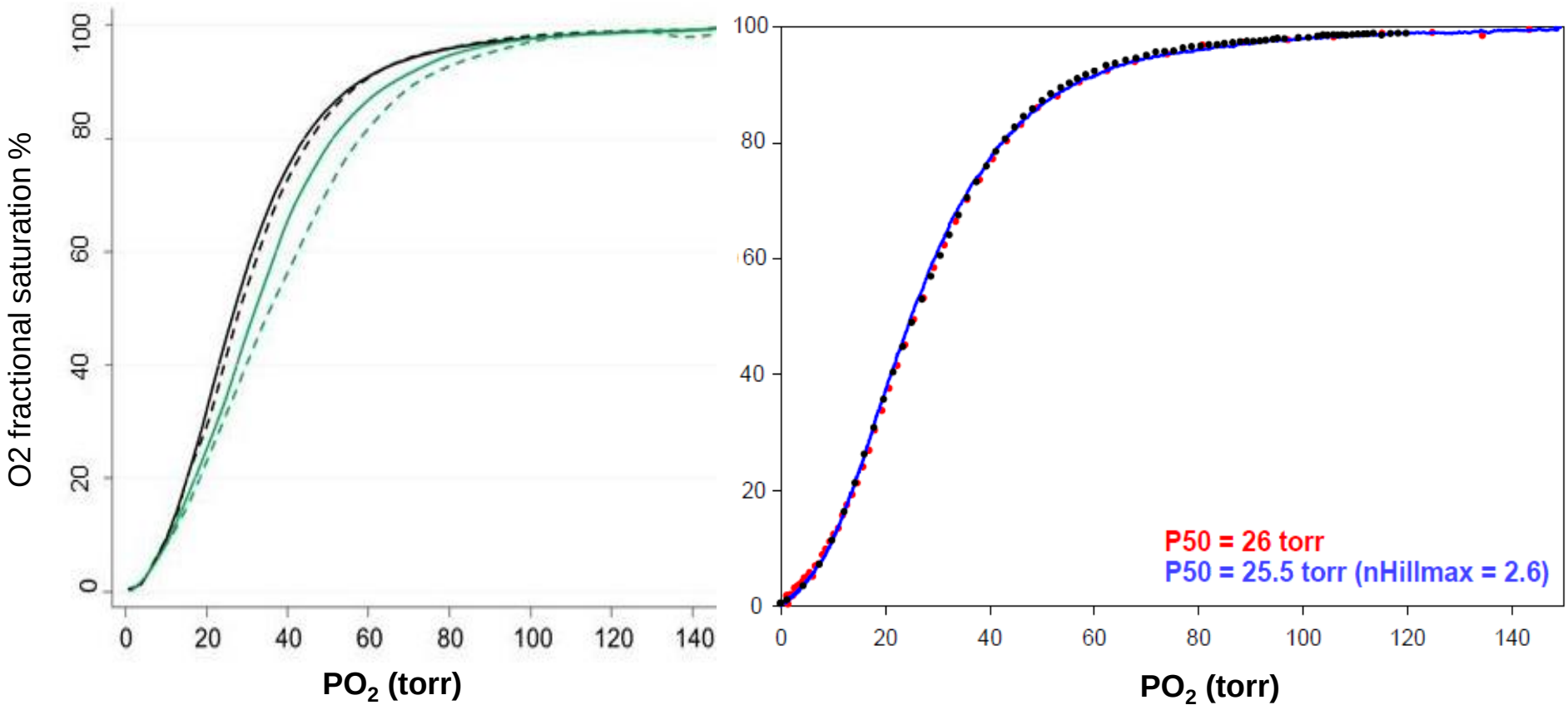


Severe SCD Subject 1204: Improvement in clinical status and hemolytic markers at 12 months

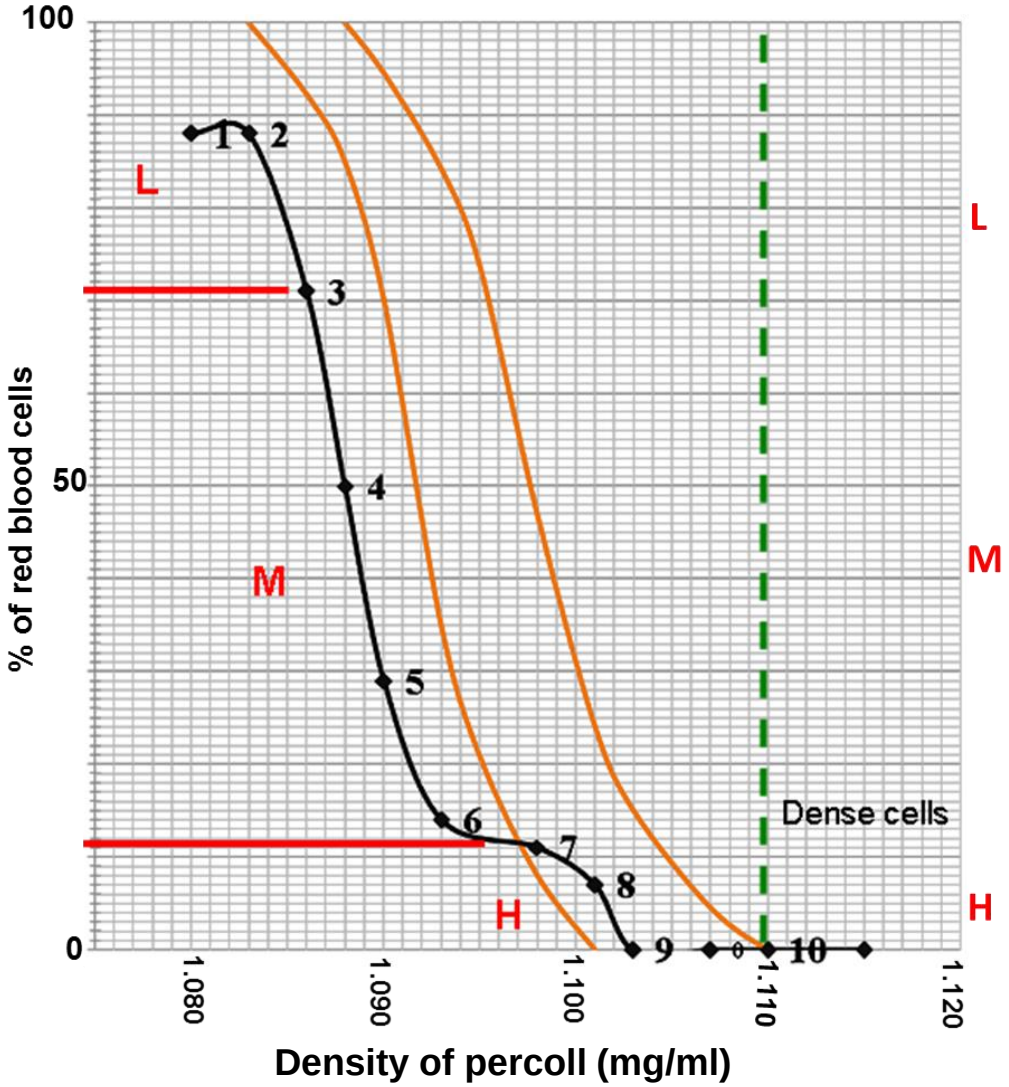
	Pre-Treatment	1 Year After Treatment
Transfusions	Chronic transfusions	**Weaned off transfusions** Last transfusion on Day + 88 (> 9 months ago)
Clinical Status	Multiple hospitalizations before starting transfusion regimen	**No hospitalizations or acute SCD-related events**
Hemolysis	Baseline reticulocyte count 238.3 x 10^9/L and LDH 626 U/L while on transfusions	• **Reticulocytes 143.1 x 10^9/L** • **LDH 274 U/L**

Data as of November 10, 2015

Severe SCD Subject 1204: Oxygen dissociation curve at 12 months functionally similar to sickle heterozygote



Severe SCD Subject 1204: Normal RBC density and normal enzymatic activity at 12 months follow-up



G6PD 11-17 U/g of Hb	PK 14-19 U/g of Hb	HK 0.74-1.14 U/g of Hb
11.8	25.6	1.5
9.3	23.8	1.4
8.9	19.4	1.2

P. Bartolucci – Henri Mondor Hospital, Creteil. G6PD: glucose-6-phosphate dehydrogenase, PK: pyruvate kinase. HK: hexokinase

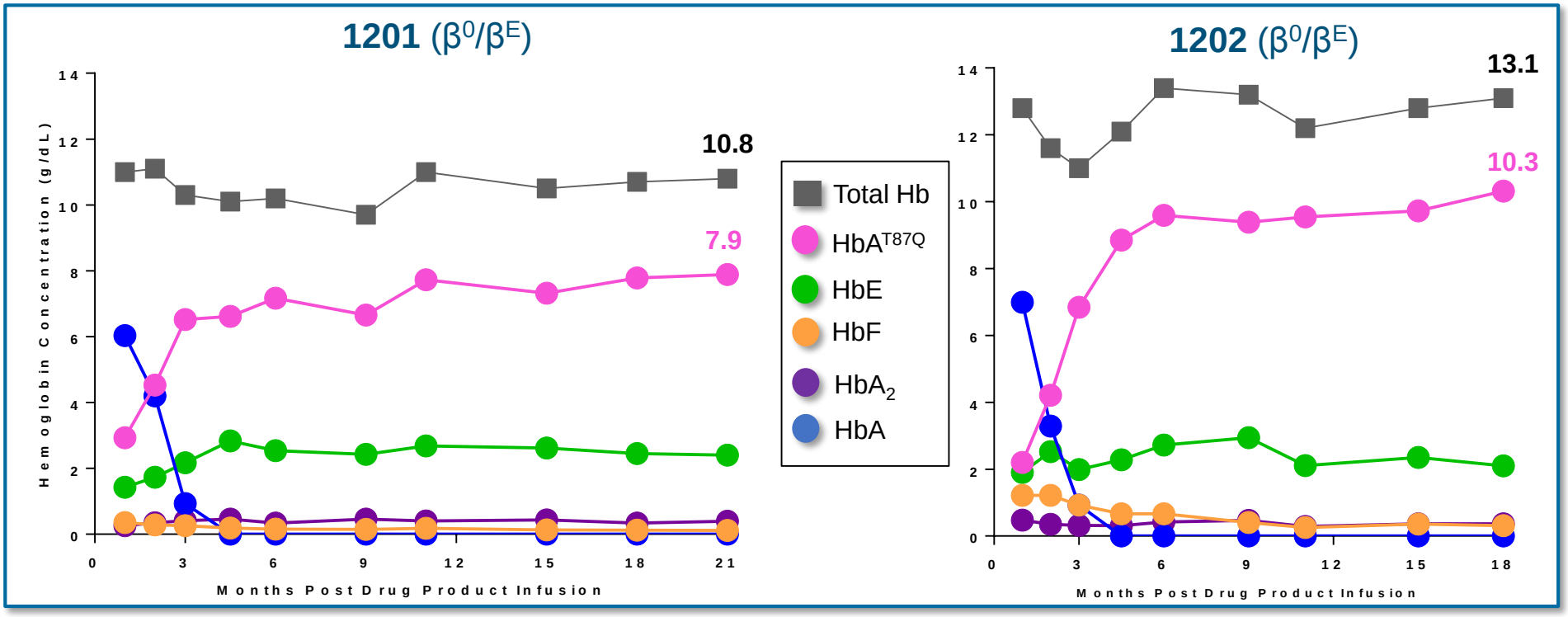
β-thalassemia major: Successful HSC harvest, drug product manufacture, and transplantation for four subjects

	1201	1202	1203	1206
Age at Consent (years)	18	16	19	17
Genotype	β^0/β^E	β^0/β^E	homozygous IVS1 nt 110 G>A	β^0/β^E
Pre-Treatment pRBC Transfusions (mL/kg/year)[1]	139	188	176	197
VCN in Drug Product[2]	1.5	2.1	0.8	1.1
CD34+ Cell Dose ($x10^6$/kg)	8.9	13.6	8.8	12.0
Follow-up period	**23.5 months**	**20.3 months**	**4.7 months**	**1.8 months**

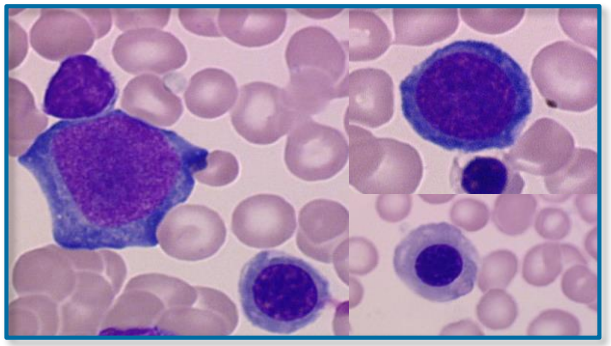
- Neutrophil engraftment Day +13 to +28, platelet engraftment Day +17 to +24
- All peri-treatment AEs consistent with myeloablative conditioning
  - Grade 3 mucositis (n=4) is the only non-hematologic Grade 3-4 AE in multiple subjects
- No drug-product related AEs
- No replication competent lentivirus (RCL) detected to date
- Highly polyclonal reconstitution without clonal dominance

[1]mean pRBC requirement per year, over the past 2 years prior to consent; [2]VCN = number of vector copies per diploid genome

β-thalassemia major: Sustained production of HbA^{T87Q} with total hemoglobin 10.8 – 13.1 g/dL

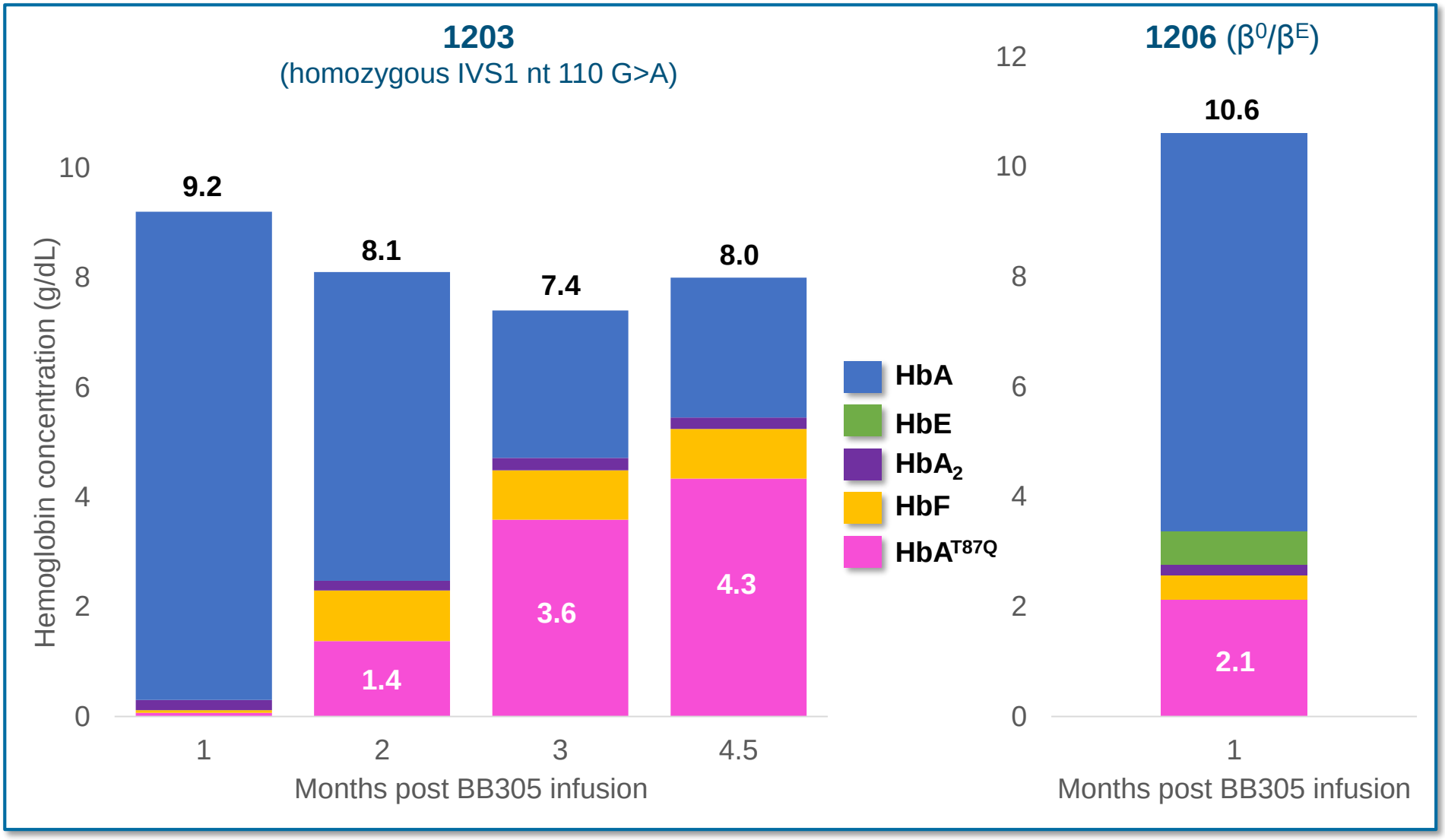


Subject 1202 bone marrow at 12 months*

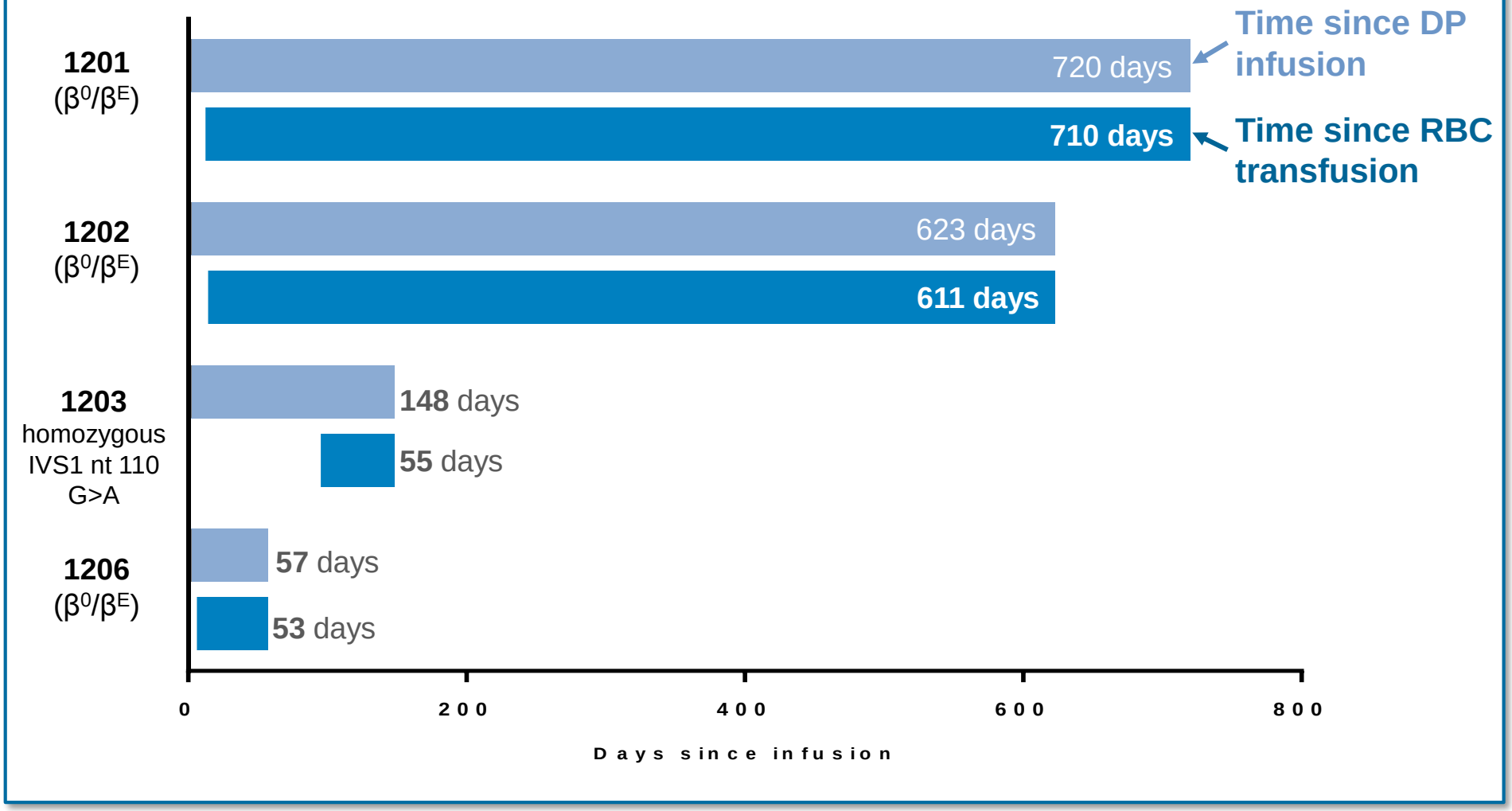


*Bone marrow sample for subject 1201 not available. Data as of November 10, 2015

β-thalassemia major: Hemoglobin levels in the two recently infused subjects



β-thalassemia major clinical results: Long-term transfusion independence in the first two subjects



Conclusions

- Study HGB-205 demonstrates continued promise of gene therapy with LentiGlobin BB305 in both severe sickle cell disease and β-thalassemia major
- Promising data in the **first subject with severe sickle cell disease treated with gene therapy**
  - Production of anti-sickling hemoglobin (50%) at 12 months well above the threshold (30%) that may show meaningful therapeutic effect
  - No further evidence of hemolysis
  - No hospitalizations, VOC, or other clinical SCD symptoms since treatment, despite weaning off transfusions
- In **β-thalassemia major**, **sustained production of HbA^{T87Q}** with stable total hemoglobin levels >10 g/dL and ongoing transfusion independence in the first two treated subjects (β^0/β^E genotype)
- Safety profile is consistent with autologous transplantation with no gene-therapy related adverse events to date and with polyclonal reconstitution

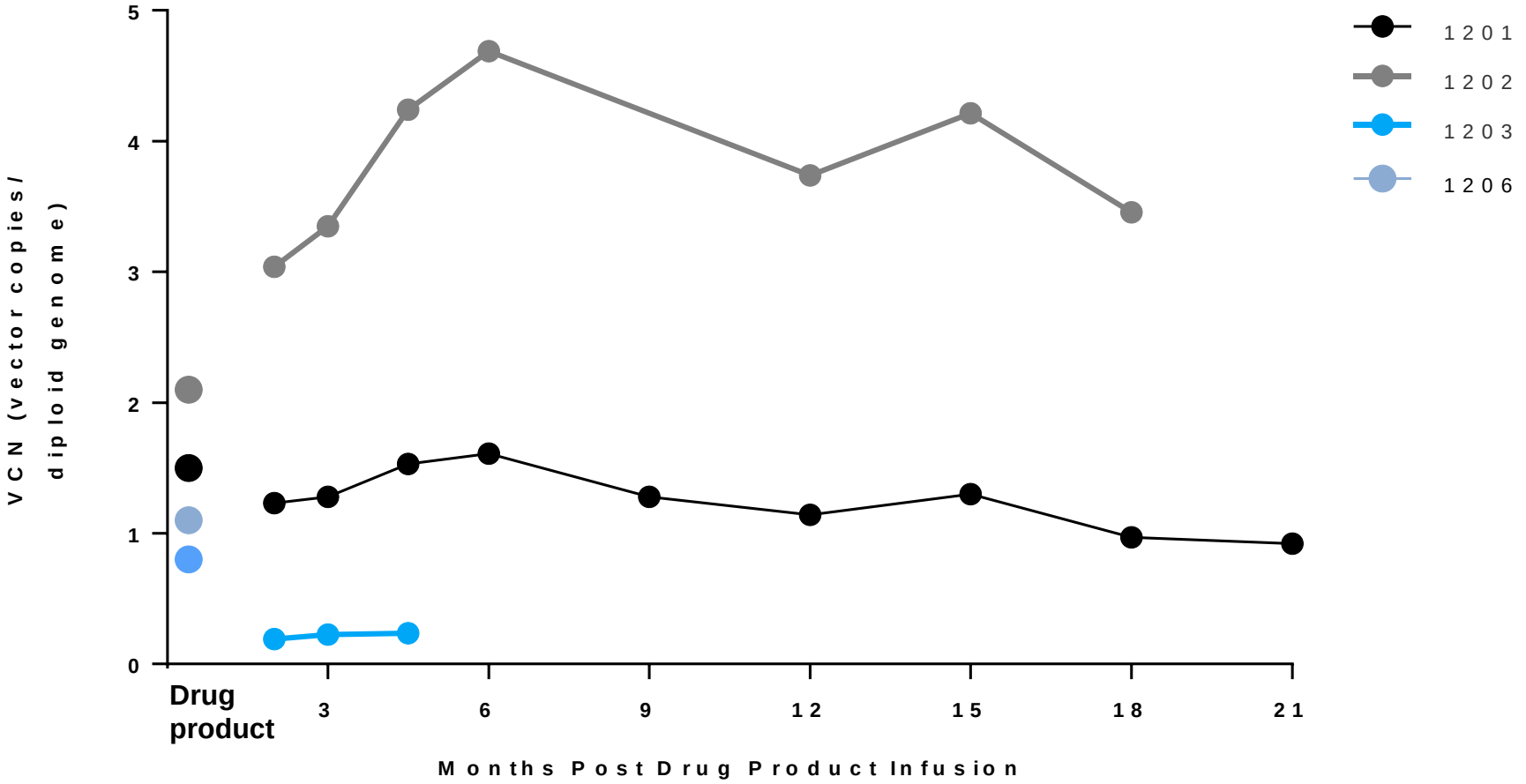
Author and collaborator affiliations

- **Hôpital Universitaire Necker - Enfants Malades, Paris, France**
- **Paris Descartes – Sorbonne Paris Cite University, Imagine Institute**
  - Marina Cavazzana
  - JA Ribeil
  - F. Touzot
  - P. Bourget
  - B. Neven
  - F. Lefrere
  - F. Suarez
  - O. Hermine
  - S. Blanche
  - M. de Montalembert
  - E. Magrin
  - L. Weber
  - P. Bartolucci
  - W. El Nemer
  - M. Semeraro
  - M.G. Rakotoson
  - L. Kriger
  - I. Guichard
- **National Center for Tumor Diseases (NCT), German Cancer Research Center (DKFZ)**
  - M. Schmidt
- **Department of Translational Oncology, National Center for Tumor Diseases, German Cancer Research Center, Heidelberg**
  - C. von Kalle
- **Centre Hospitalier de Nice Sophia-Antipolis**
  - F. Monpoux
- **CEA, Institut of Emerging Diseases and Innovative Therapies and University of Paris-Sud, Fontenay-aux-Roses, France (and also Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA, and Mahidol University and Ramathibodi Hospital, Bangkok, Thailand)**
  - Philippe Leboulch
  - E. Payen
  - Y. Beuzard
  - S. Chretien
  - R. Cavallesco
- **Centre Hospitalier Intercommunal de Créteil (CHIC)**
  - C. Pondarre
- **Children's Hospital Oakland Research Institute**
  - F. Kuypers
- **Hôpital Universitaire Necker - Enfants Malades, Paris, France and Groupe Hospitalier Universitaire Paris-Sud**
  - S. Hacein-Bey-Abina
- **bluebird bio, Inc.**
  - L. Sandler
  - S. Soni

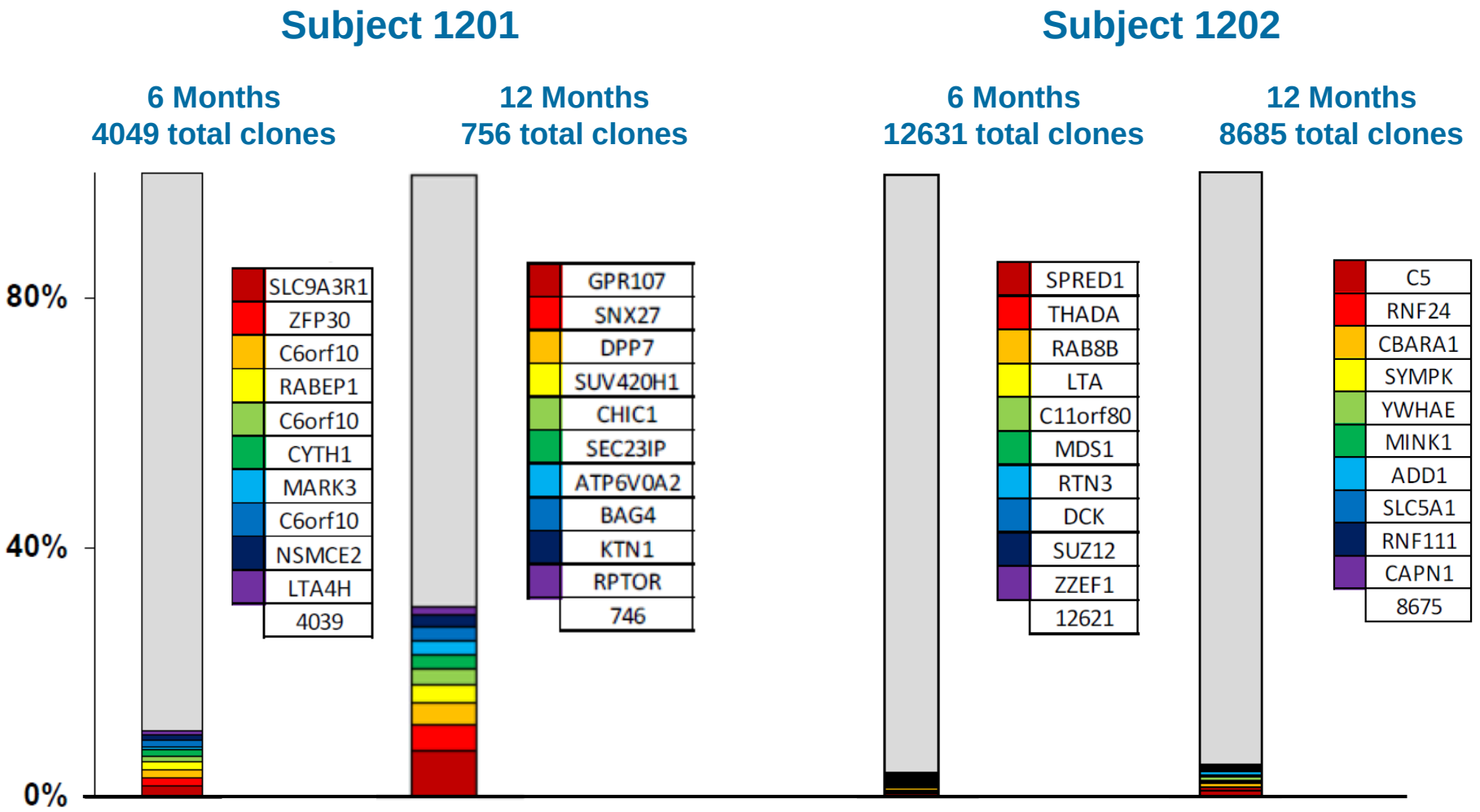
Most importantly, we wish to thank all the patients and their families

Back-Up

β-thalassemia major: Vector copy number in drug product and peripheral blood leukocytes



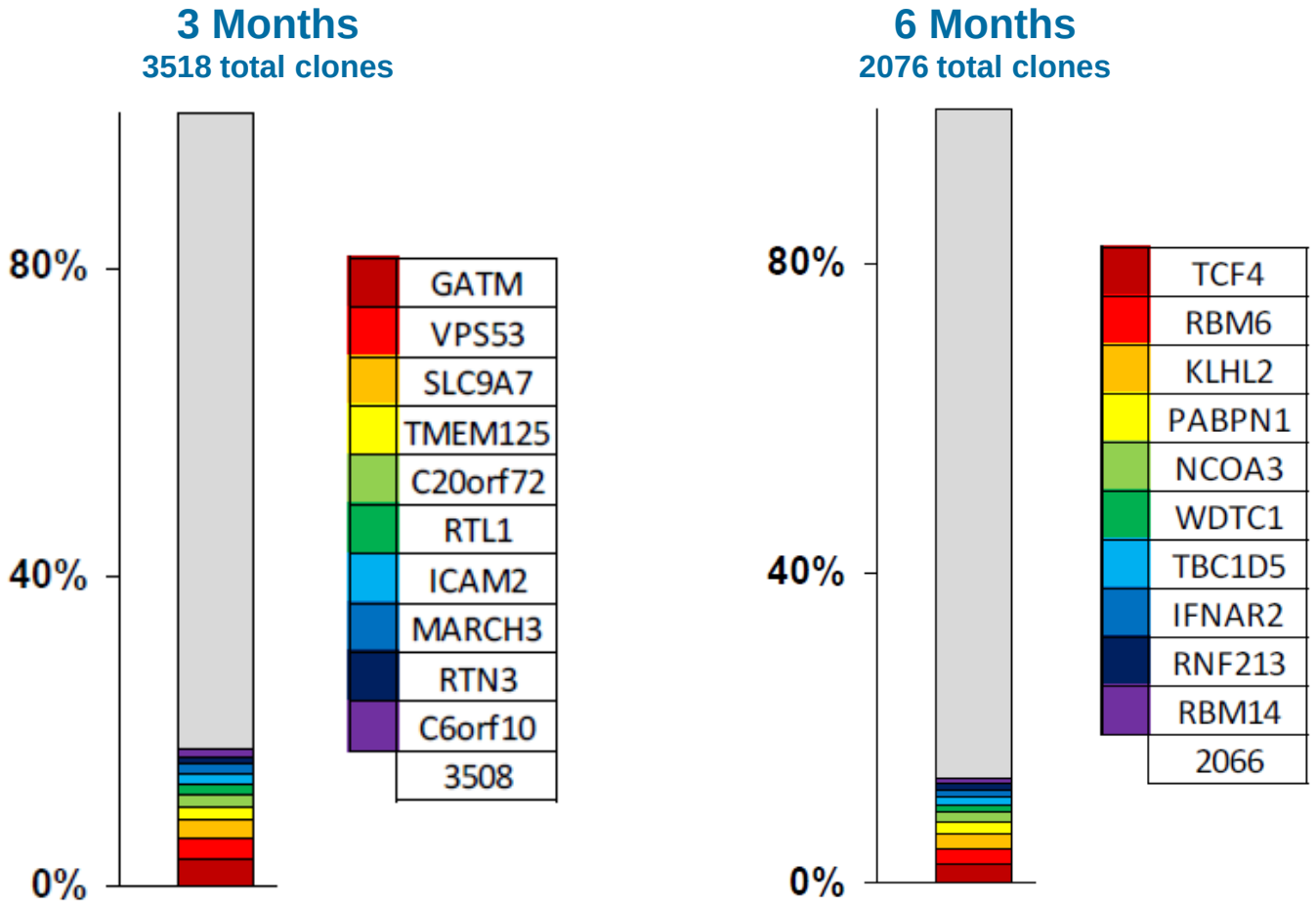
β-thalassemia major: Highly polyclonal repopulation in peripheral blood leukocytes to 12M



No clonal dominance detected

Severe SCD: Highly polyclonal repopulation in peripheral blood leukocytes

Subject 1204



No clonal dominance detected