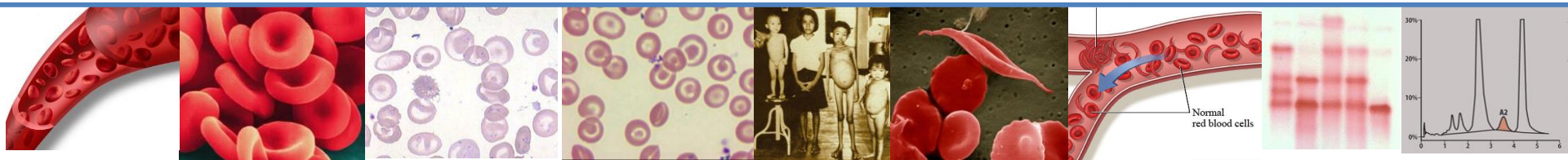
Gene therapy for the treatment of β-hemoglobinopathies

Prof. Philippe Leboulch
University of Paris - CEA, and Harvard Medical School – Brigham & Women's Hospital
Hämatologie Heute, 21 March 2019, Köln, Germany

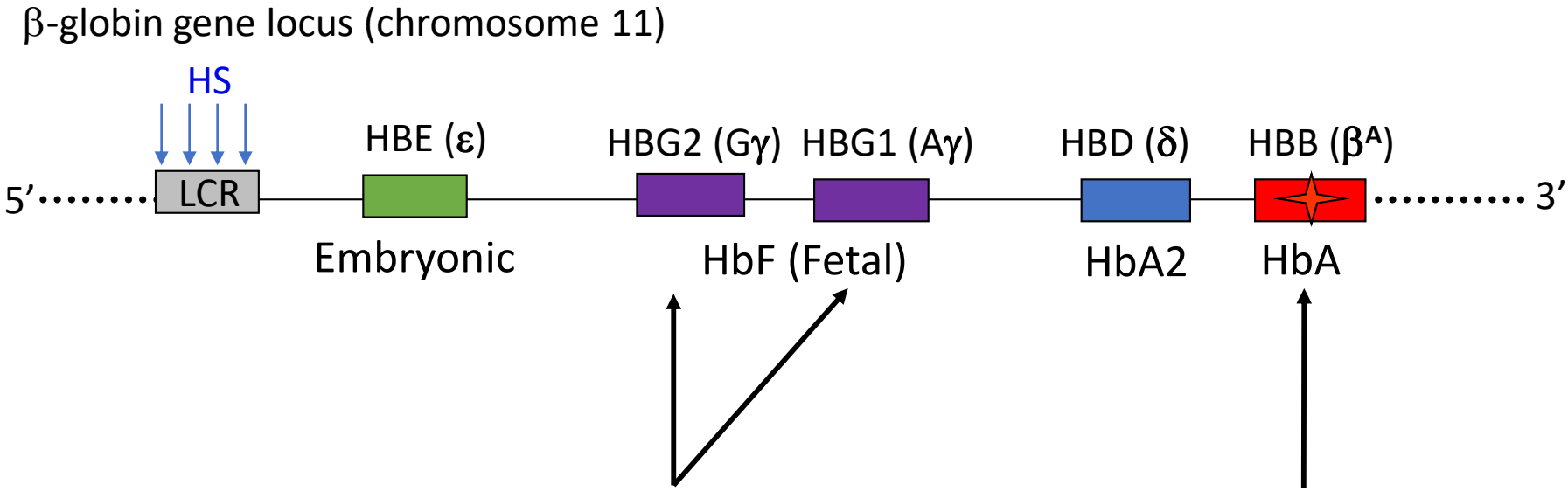


DISCLOSURE:

Co-founder of *bluebird bio* and *c*o-chair of *bluebird bio*'s SAB

The products reported here remain EXPERIMENTAL

Approaches to the gene therapy of β-globin disorders



Gene replacement/addition

β-globin

γ/β-globin

Marked and anti-sickling $\beta^{A\text{-}T87Q}$-globin

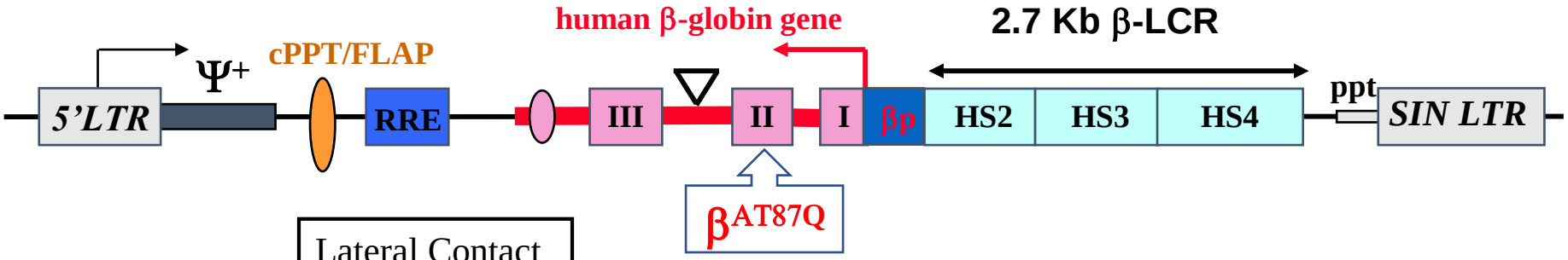
Genome editing (deletion)

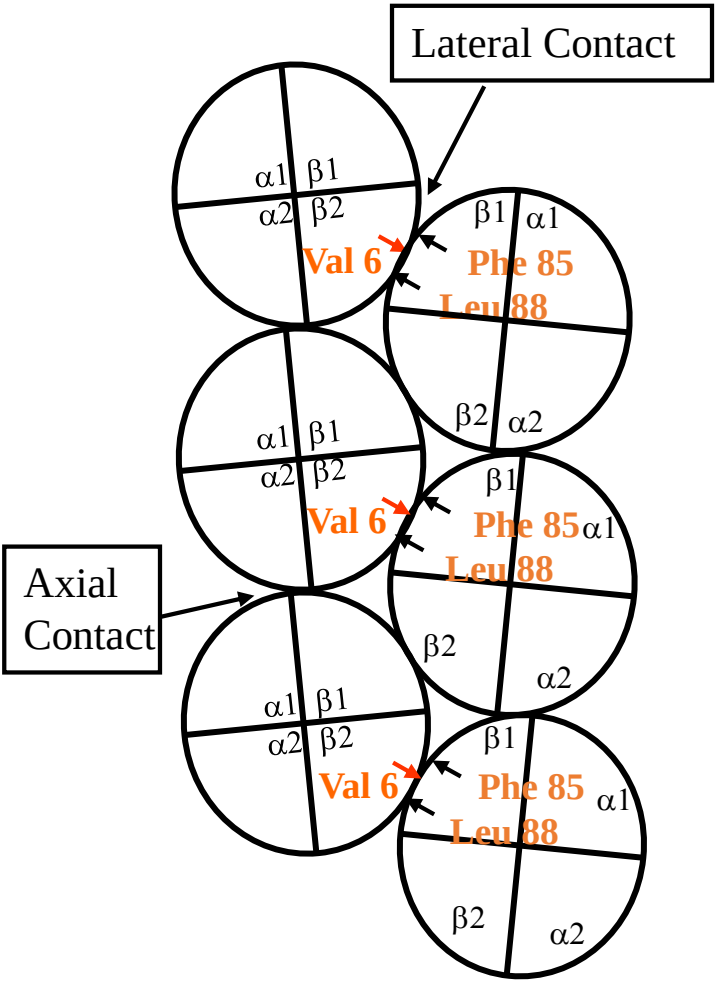
Reactivation of γ-globin

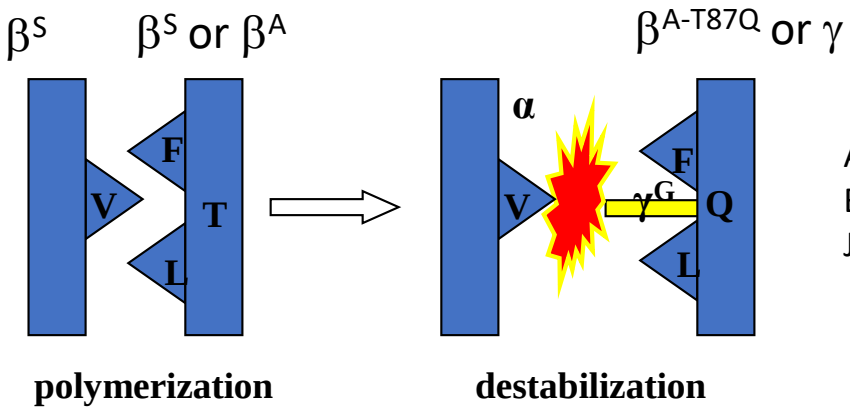
Genome editing (repair)

- 200 mutations causing β-thalassemia
- Efficacy levels in hematopoietic stem cells
- Concerns about p53 -/- selection
- Concerns about off-target effects
- Concerns about untoward "Indels"

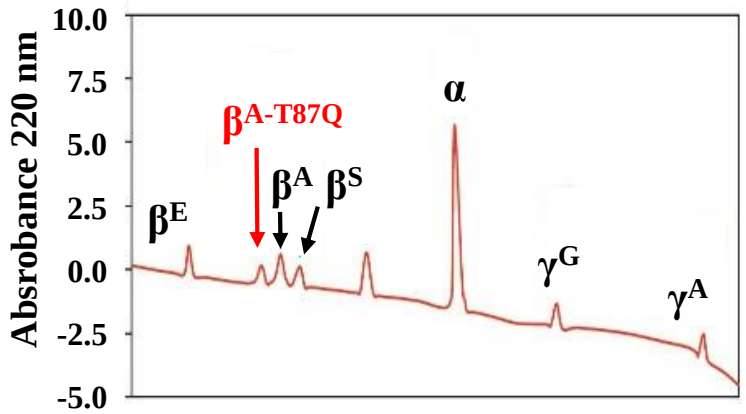
LentiGlobin $\beta^{A\text{-}T87Q}$ vector – rationale for use in SCD and β-thalassemia







Adachi et al.
Blood 1996
JBC 1996



14 DECEMBER 2001 VOL 294 SCIENCE

Correction of Sickle Cell Disease in Transgenic Mouse Models by Gene Therapy

Robert Pawliuk,[1,2] Karen A. Westerman,[1,2] Mary E. Fabry,[3] Emmanuel Payen,[4] Robert Tighe,[1,2] Eric E. Bouhassira,[3] Seetharama A. Acharya,[3] James Ellis,[5] Irving M. London,[1,6] Connie J. Eaves,[7] R. Keith Humphries,[7] Yves Beuzard,[4] Ronald L. Nagel,[3] Philippe Leboulch,[1,2,4,8]*

- Pre-clinical studies
- Large-scale GMP manufacturing
- Approval regulatory authorities
- Patients' inclusion

First clinical trial approved worldwide for the use of lentiviral vectors

Proof of principle of transfusion-independence in a patient

nature Vol 467|16 September 2010|doi:10.1038/nature09328

LETTERS

nature *Published Sept 2010*

Transfusion independence and *HMGA2* activation after gene therapy of human β-thalassaemia

Marina Cavazzana-Calvo[1,2]*, Emmanuel Payen[3,4,5]*, Olivier Negre[3,4,5,6], Gary Wang[7], Kathleen Hehir[8], Floriane Fusil[3,4,5], Julian Down[8], Maria Denaro[8], Troy Brady[7], Karen Westerman[8,9], Resy Cavallesco[9], Beatrix Gillet-Legrand[6], Laure Caccavelli[1,2], Riccardo Sgarra[10], Leila Maouche-Chrétien[3,4], Françoise Bernaudin[11], Robert Girot[12], Ronald Dorazio[8], Geert-Jan Mulder[8], Axel Polack[8], Arthur Bank[13], Jean Soulier[5], Jérôme Larghero[5], Nabil Kabbara[5], Bruno Dalle[5], Bernard Gourmel[5], Gérard Socie[5], Stany Chrétien[3,4,9], Nathalie Cartier[14], Patrick Aubourg[14], Alain Fischer[1,2], Kenneth Cornetta[15], Frédéric Galacteros[16], Yves Beuzard[3,4,5], Eliane Gluckman[5], Frederick Bushman[7], Salima Hacein-Bey-Abina[1,2]* & Philippe Leboulch[3,4,9]*

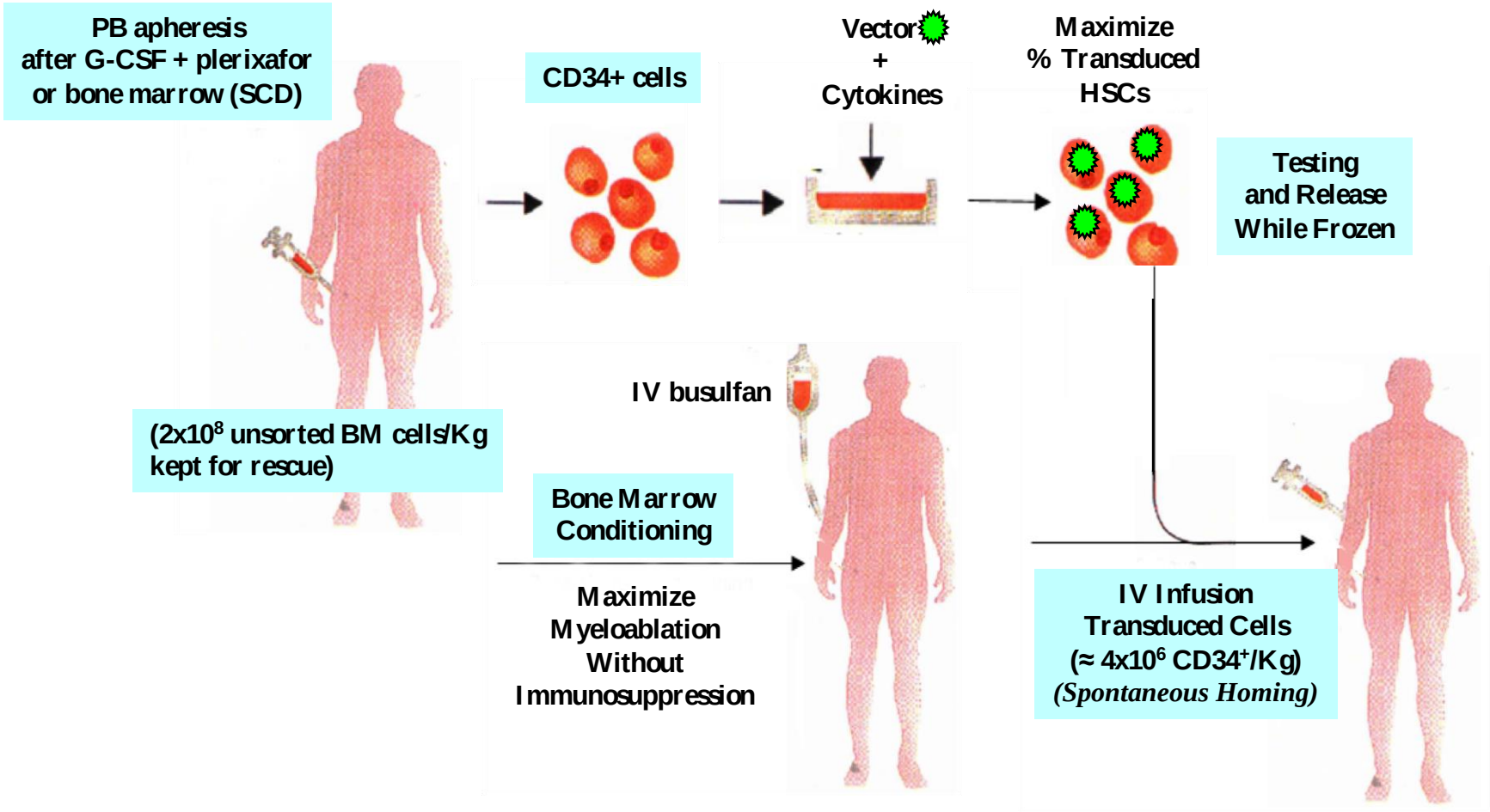
further optimized vector LentiGlobin BB305 (Leboulch *et al.* and *bluebird bio*)

~ 60 patients treated to date

Overview of the clinical protocol

Key eligibility:

- β-Thal major (≥ 100 mL pRBCs / kg / year) or severe SCD
- No HLA-matched sibling donor



bluebird bio Phase I/II trials with $\beta^{A\text{-}T87}$-LentiGlobin

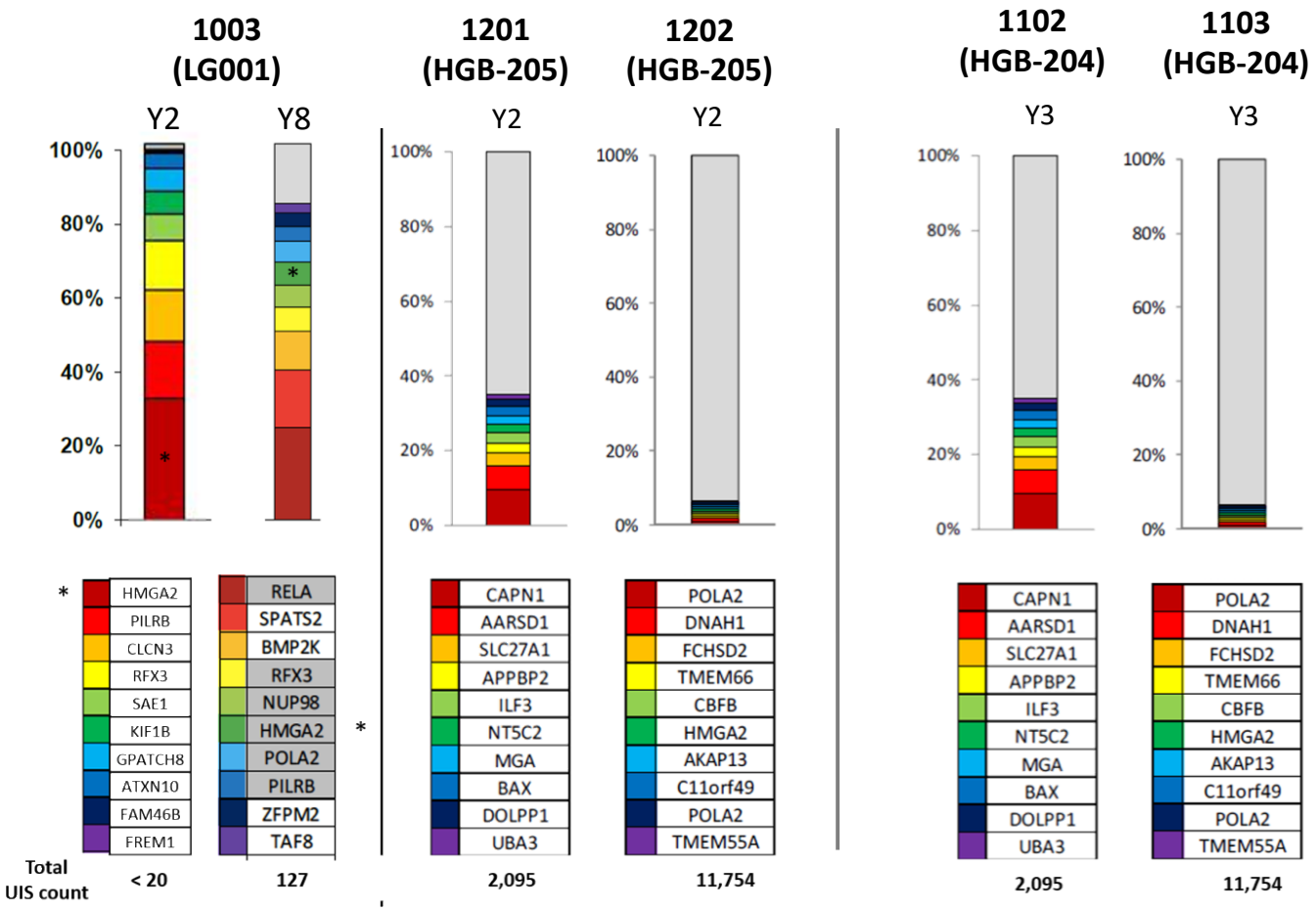
- Integrated 2nd generation $\beta^{A\text{-}T87}$-LentiGlobin vector ("**BB305**") exactly identical to 1st generation vector (Leboulch and coll. *Science* 2001, *Nature* 2010), except for lack of insulator (at regulators' request).
- Enhanced vector purification (*bluebird bio*) after CMV-based packaging using same packaging system (Leboulch lab).
- Open label, single arm studies

Study	Centers	Indication	Planned subjects	Current Status *(September 14, 2018)*
HGB-205 (France)	1 in France	β-thalassemia major (TDT) and severe SCD 5-35 yo	**7**	**COMPLETED** (3 βE/β0, 1 β0/0 like and 3 SCD (SS and S0)) (longest follow-up > 5 years)
HGB-204 "Northstar Study" (USA)	4 in US 1 in France 1 in Australia 1 in Thailand	β-thalassemia major (TDT) 12-35 yo	**18**	**COMPLETED** (including 8 β0/0) (longest follow-up > 4 years)
HGB-206 (USA)	3-6 in US	Severe SCD >18 yo	**29**	**10+ subjects treated** (longest follow-up > 3 years)

Ongoing *bluebird bio* Phase III trials with $\beta^{A\text{-}T87}$-LentiGlobin

Study	Centers	Indication	Planned subjects	Current Status *(September 14, 2018)*
HGB-207 "Northstar-2 Study" (USA-EU) Pivotal (US) /confirmatory (EU)	3 in US 1 in Thailand 7 in Europe *(France, UK, Germany, Italy, Greece)* **Primary Endpoint:** **Transfusion Independence** Weighted average Hb ≥ 9 g/dL without any transfusions for ≥ 12 months	**Non-$\beta^{0/0}$** β-thalassemia major (TDT) <50 yo	**23**	**16 subjects treated** **Median follow-up: 9.3 months** (min – max: 0.7 – 20.4)
HGB-212 "Northstar-3 Study" (USA-EU)	As above **Primary Endpoint:** **Transfusion Reduction** ≥ 60% reduction in transfused RBC volume 12 – 24 months post-DP infusion compared to the 24 months pre-DP infusion **Key secondary endpoint:** **Transfusion Independence** Weighted average Hb ≥ 9 g/dL without any RBC transfusions for ≥ 12 months	**$\beta^{0/0}$** β-thalassemia major (TDT) <50 yo	**15**	**3 subjects treated** **Median follow-up: 4.2 months** (min – max: 1.4 – 9.2)

No vector-related safety events in any of the trial patients*:
Highly polyclonal repopulation for all β-Thal and SCD patients



- No clonal dominance detected
- Maximum single clone contribution <8% of total clonality

**1 patient with Grade 3 AE of thrombocytopenia possibly related to LentiGlobin in Phase 3*

Transfusion-dependent β-Thalassemia

Results of completed Phase I/II trial HGB-204

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 19, 2018 VOL. 378 NO. 16

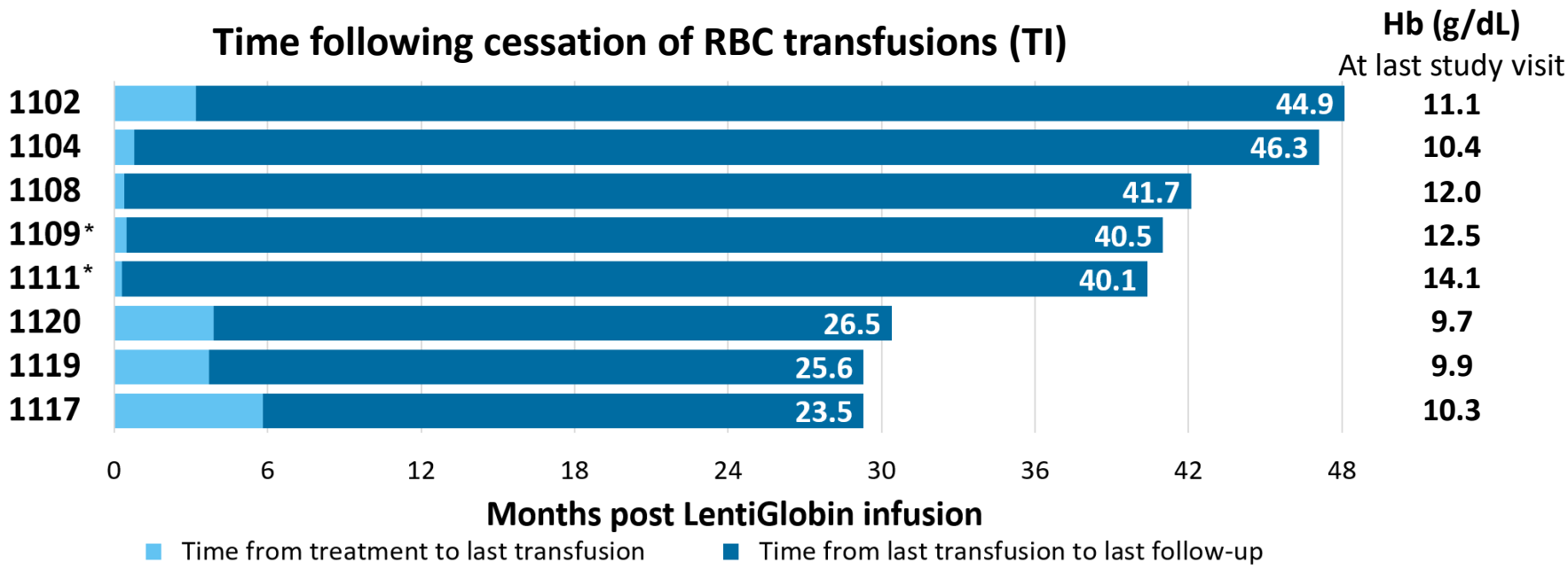
Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia

A.A. Thompson, M.C. Walters, J. Kwiatkowski, J.E.J. Rasko, J.-A. Ribeil, S. Hongeng, E. Magrin, G.J. Schiller, E. Payen, M. Semeraro, D. Moshous, F. Lefrere, H. Puy, P. Bourget, A. Magnani, L. Caccavelli, J.-S. Diana, F. Suarez, F. Monpoux, V. Brousse, C. Poirot, C. Brouzes, J.-F. Meritet, C. Pondarré, Y. Beuzard, S. Chrétien, T. Lefebvre, D.T. Teachey, U. Anurathapan, P.J. Ho, C. von Kalle, M. Kletzel, E. Vichinsky, S. Soni, G. Veres, O. Negre, R.W. Ross, D. Davidson, A. Petrusich, L. Sandler, M. Asmal, O. Hermine, M. De Montalembert, S. Hacein-Bey-Abina, S. Blanche, P. Leboulch, and M. Cavazzana

Extended to last data cut off = September 14, 2018

Drug Product Characteristics	N = 18 median (min – max)
Drug product cell dose CD34+ cells x10^6/kg	**8.1** (5.2 – 18.1)
Drug product VCN[†] vector copies/diploid genome	**0.7** (0.3 – 1.5)
CD34+ cells transduced[†] %	**31.5** (17 – 58)
Treatment Characteristics	
Neutrophil engraftment[#] study day	**18.5** (14 – 30)
Platelet engraftment[^] study day	**39.5** (19 – 191)

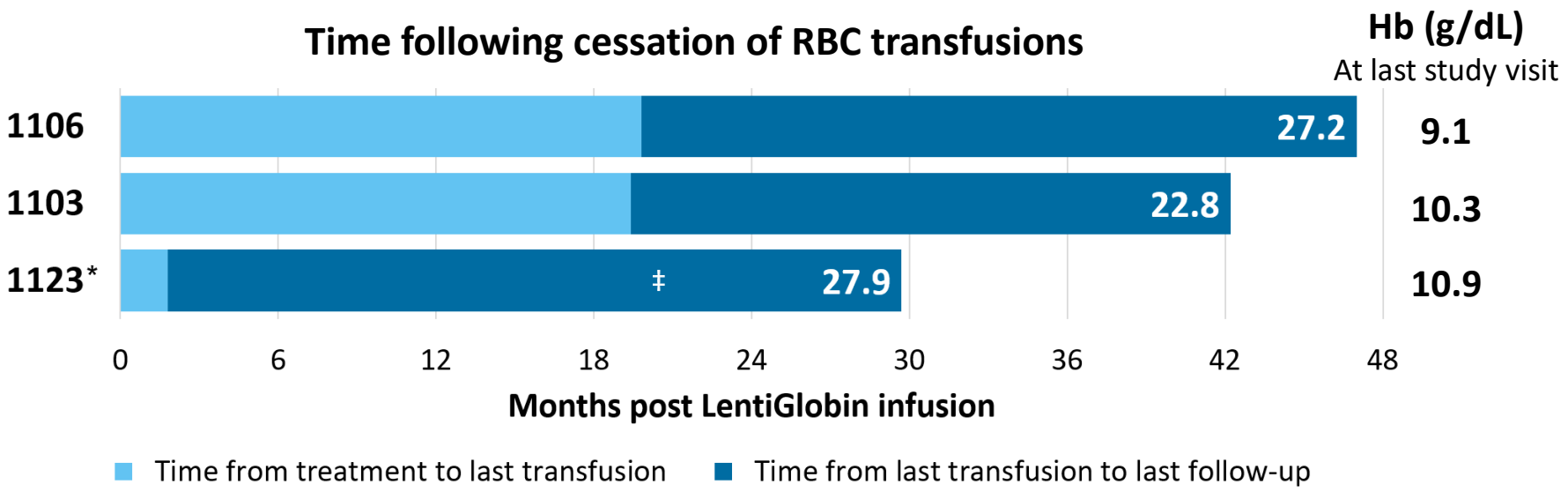
8/10 patients with non-β^0/β^0 genotypes have achieved sustained transfusion-independence *(failures linked to low VCN)*



*Indicates male patients. Hb, hemoglobin; TI, transfusion independence (weighted average Hb ≥9 g/dL without any red blood cell transfusions for ≥12 months)

Median duration of sustained TI: 38.0 months (min – max: 21.2 – 43.6 months
Median weighted average Hb during TI: 10.2 g/dL (min – max: 9.3 – 13.2 g/dL)

3/8 patients with β^0/β^0 genotype have achieved sustained transfusion-independence



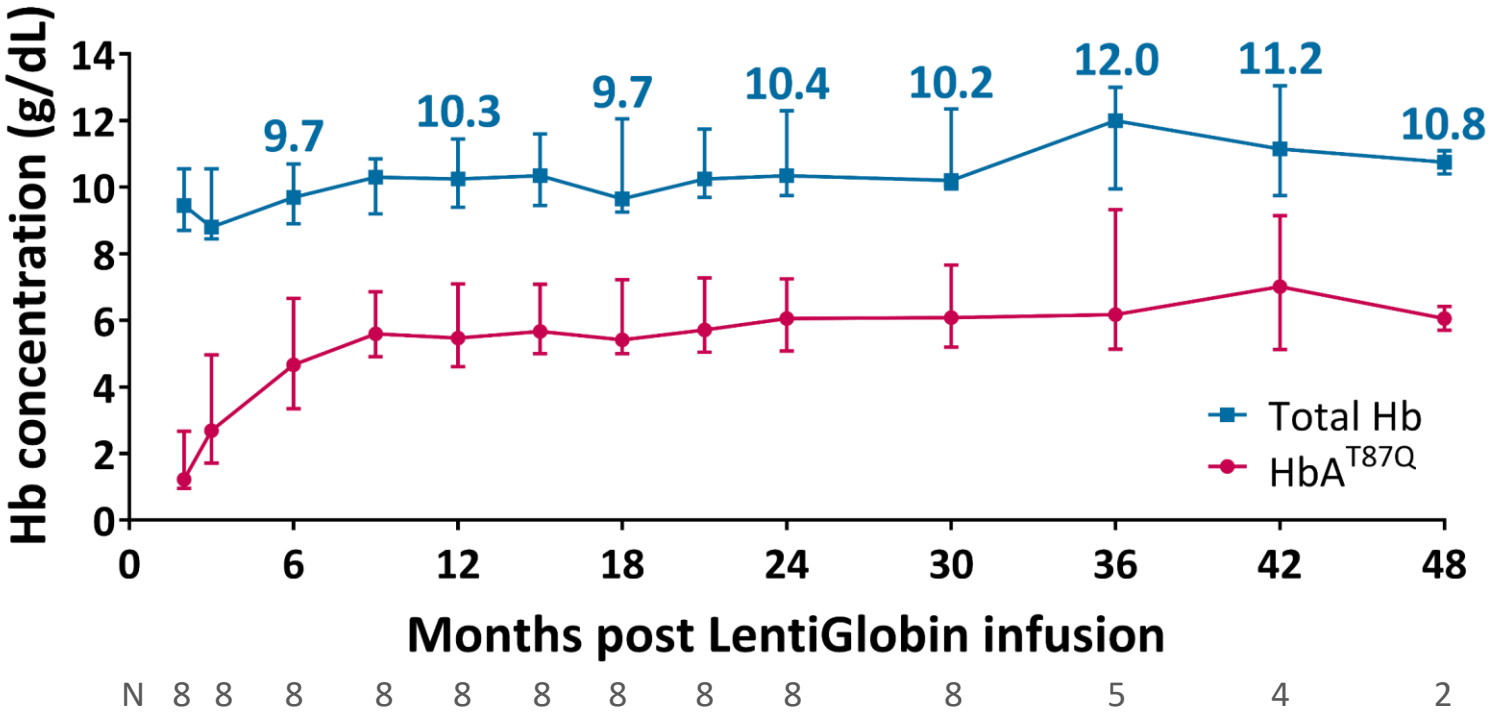
Median duration of sustained TI: 16.4 months (min – max: 16.1 – 20.8 months)
Median weighted average Hb during TI: 9.9 g/dL (min – max: 9.5 – 10.1 g/dL)

*Indicates male patient
‡Patient had a single transfusion for an acute event of cat scratch disease
Hb, hemoglobin; TI, transfusion independence (weighted average Hb ≥9 g/dL without any red blood cell transfusions for ≥12 months)

Transfusion-free $Hb^{A\text{-}T87Q}$ and total Hb levels in blood are stable after LentiGlobin gene therapy

I. in non-β^0/β^0 genotypes

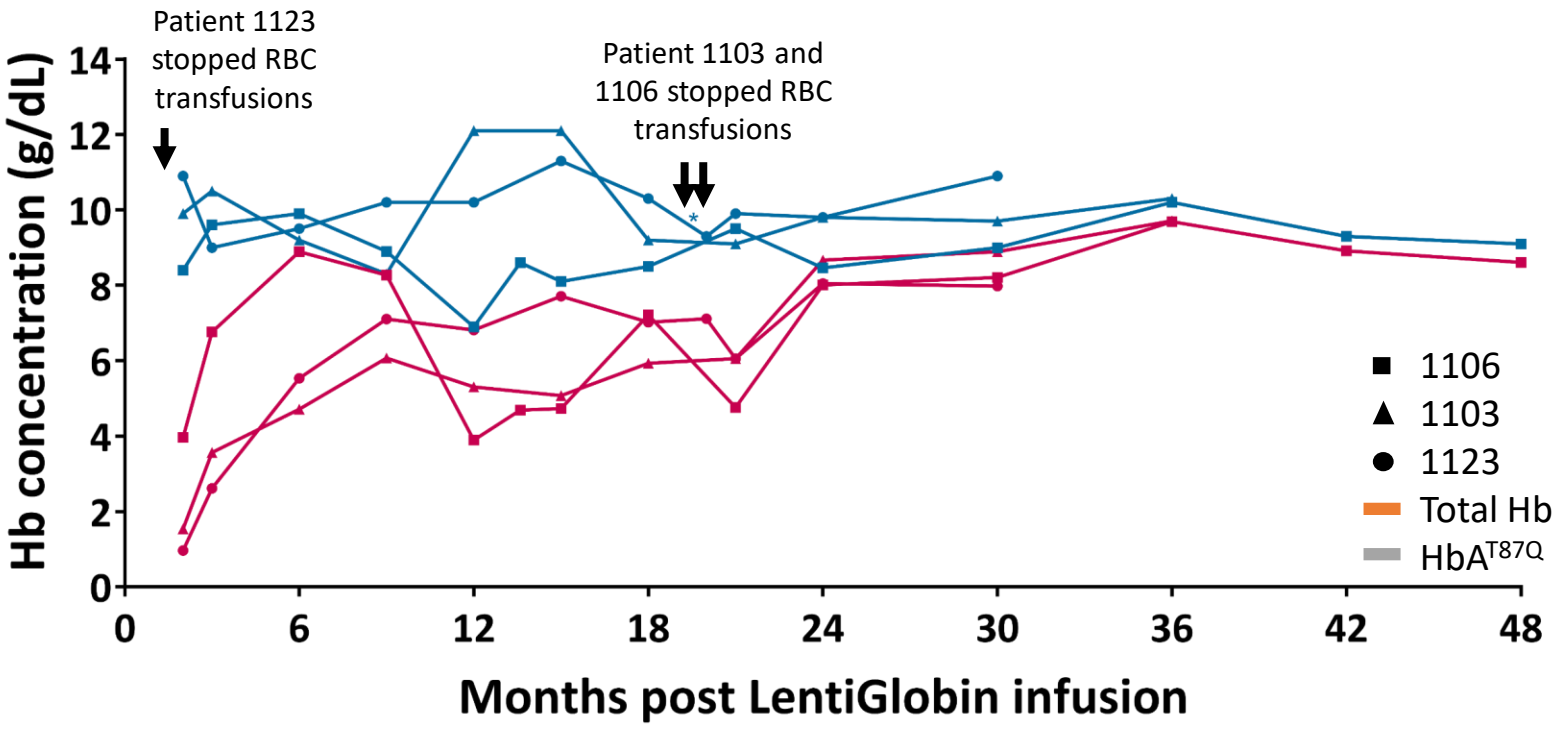
Median Hb in patients with non-β^0/β^0 genotypes who achieved transfusion independence



Medians (Q1, Q3) depicted; Hb, hemoglobin

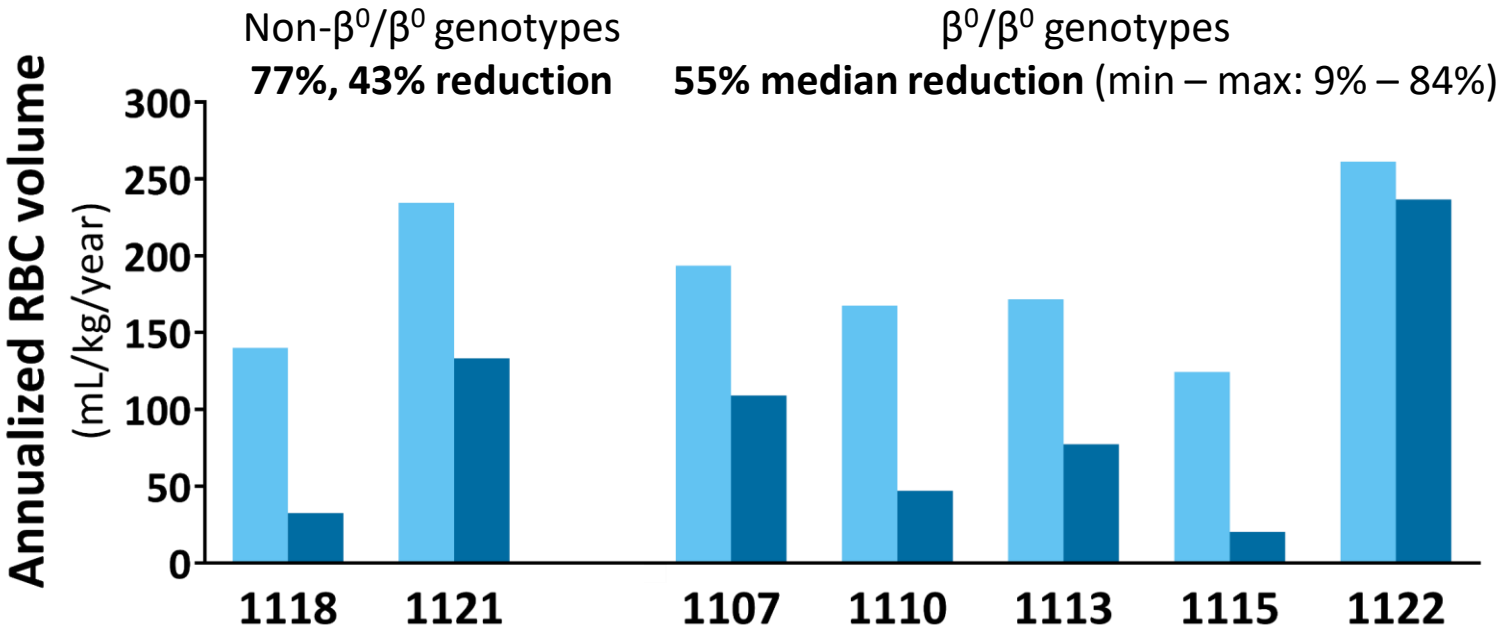
Transfusion-free $Hb^{A\text{-}T87Q}$ and total Hb levels in blood are stable after LentiGlobin gene therapy

II. in β^0/β^0 genotype



*Patient 1123 had a single transfusion for an acute event of cat scratch disease

Reduction in annualized RBC transfusion volumes in patients still receiving transfusions



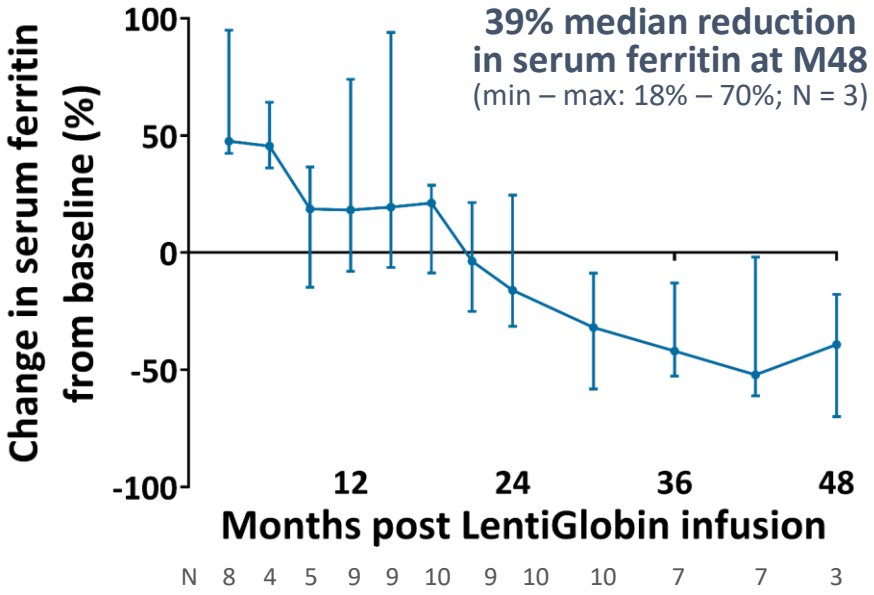
Pre-treatment: Annualized volume of RBC transfusions in the 2 years prior to study enrollment

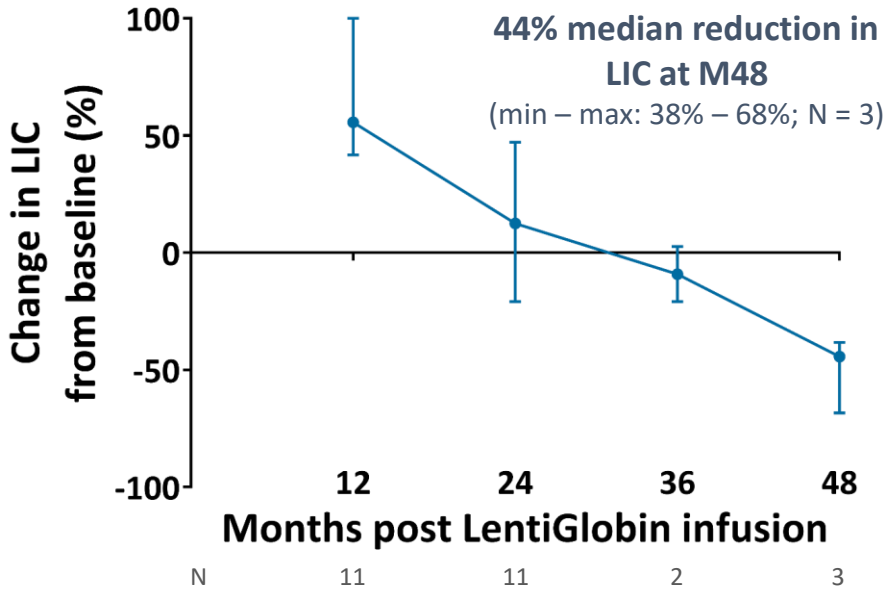
Post-treatment: Annualized on-study volume of RBC transfusions starting at month 6 post-DP infusion through last study visit

RBC, red blood cell

Reduction in iron overload after LentiGlobin gene therapy

% Change in serum ferritin and LIC from baseline in patients who achieved TI





Patients re-initiated iron chelation therapy a median of 13 months after LentiGlobin infusion (min – max: 2 – 16 months)

Medians (Q1, Q3) depicted. One patient did not have a baseline serum ferritin level. LIC, liver iron concentration; TI, transfusion independence

Results of completed Phase I/II trial HGB-205

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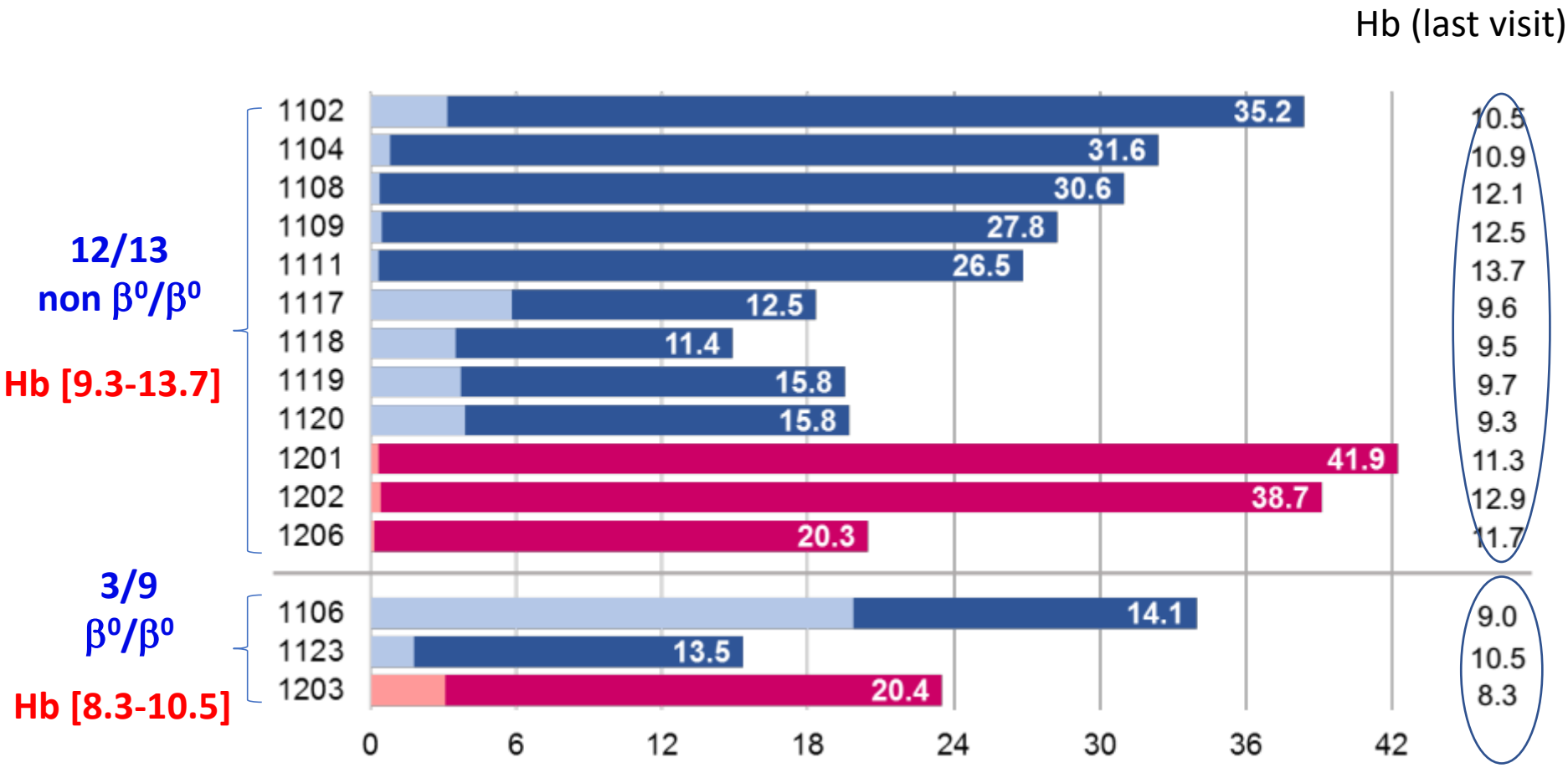
Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia

A.A. Thompson, M.C. Walters, J. Kwiatkowski, J.E.J. Rasko, J.-A. Ribeil, S. Hongeng, E. Magrin, G.J. Schiller, E. Payen, M. Semeraro, D. Moshous, F. Lefrere, H. Puy, P. Bourget, A. Magnani, L. Caccavelli, J.-S. Diana, F. Suarez, F. Monpoux, V. Brousse, C. Poirot, C. Brouzes, J.-F. Meritet, C. Pondarré, Y. Beuzard, S. Chrétien, T. Lefebvre, D.T. Teachey, U. Anurathapan, P.J. Ho, C. von Kalle, M. Kletzel, E. Vichinsky, S. Soni, G. Veres, O. Negre, R.W. Ross, D. Davidson, A. Petrusich, L. Sandler, M. Asmal, O. Hermine, M. De Montalembert, S. Hacein-Bey-Abina, S. Blanche, P. Leboulch, and M. Cavazzana

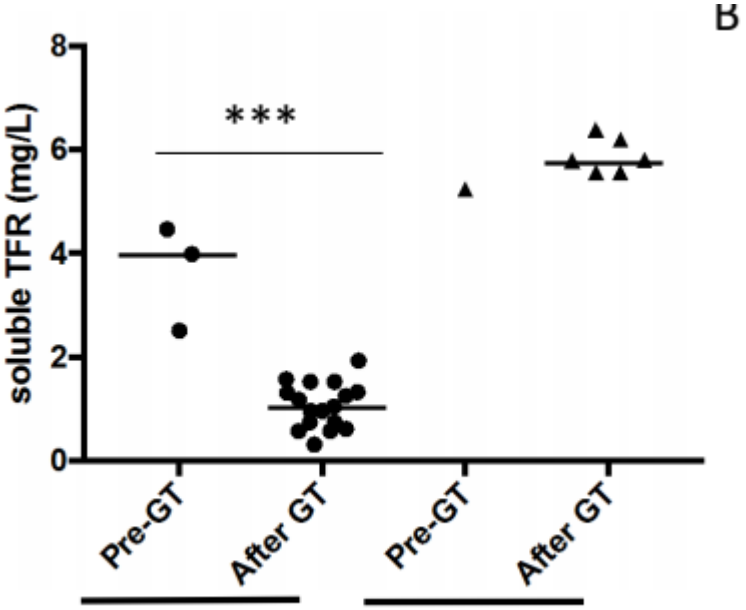
Last data cut off = June 02, 2017 (trends stable post trial)

All patients with non-β^0/β^0 genotypes (N=3) or β^0/β^0-equivalent (N=1) genotype are transfusion free *(in RED here below)*



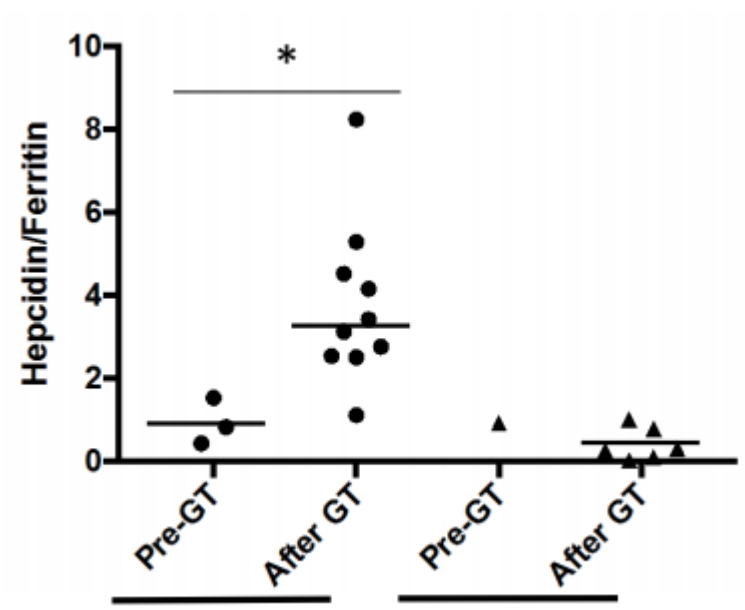
trends stable post trial

Normalization of markers of erythroid expansion in patients with Hb > 10g/dL



1201,1202, 1206 Hb=[11.3-12.9]

1203 Hb=[8.3]



1201,1202, 1206 Hb=[11.3-12.9]

1203 Hb=[8.3]

Several patients have discontinued iron chelation therapy

Interim results of Phase III trial HGB-207

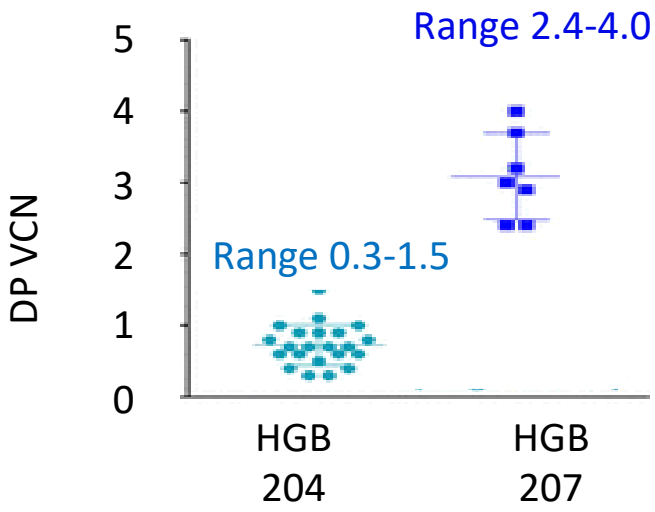
HGB-207

Phase 3, multi-center, global study
NCT02906202

- Non-β^0/β^0 genotypes
- N = 23 patients ≤ 50 years of age
- Ongoing

Last data cut off = September 14, 2018

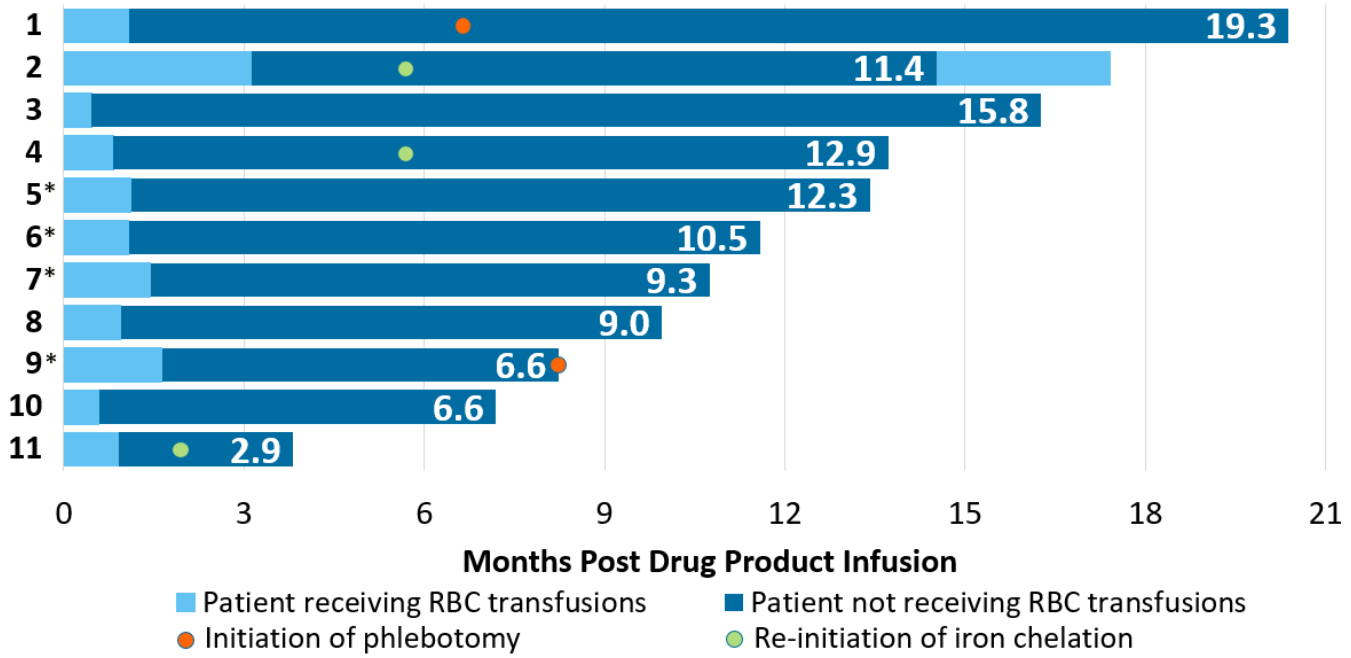
Improved DP manufacturing



Drug Product Characteristics	median (min – max)
Drug product cell dose CD34+ cells x 10^6/kg	**7.7** (5.0 – 19.4)
Drug product VCN[†] vector copies/diploid genome	**3.1** (2.1 – 5.6)
CD34+ cells transduced[^] %	**81** (53 – 90)
Treatment Characteristics	
Busulfan AUC[‡] µM*min	**4545** (3709 – 8947)
Neutrophil engraftment[#] study day	**19** (13 – 32)
Platelet engraftment[§] study day	**44.5** (20 – 84)

10/11 patients with non-β^0/β^0 genotypes are transfusion free with Hb > 11 g/dL

Time free from chronic transfusions in patients with ≥ 3 months follow-up

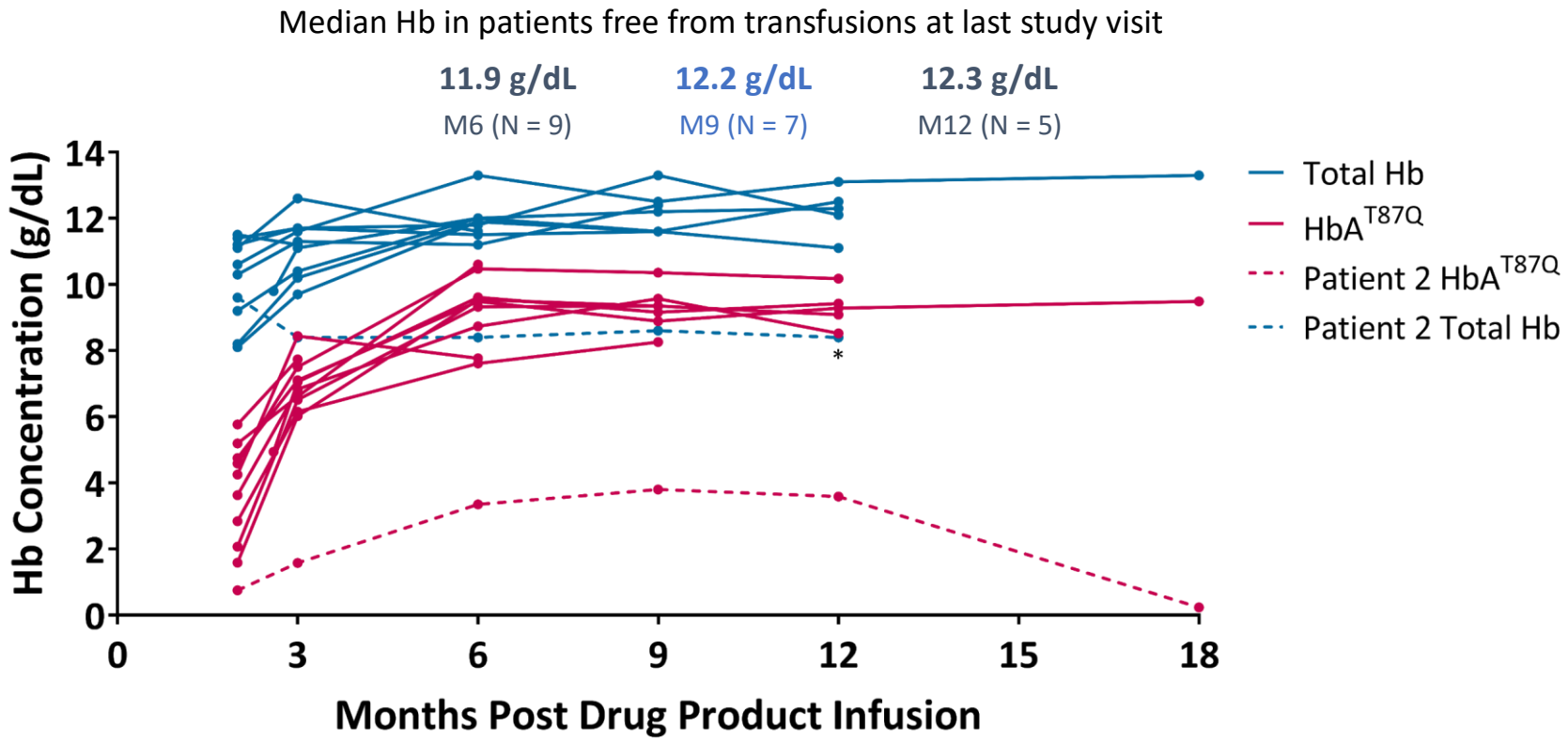


	Hb (g/dL)	Peripheral VCN
	At last study visit	
1	13.3	3.2
2	11.3‡	0.4
3	11.1	2.2
4	12.1	1.7
5*	12.3	1.7
6*	12.5	2.9
7*	11.6	5.6
8	12.4	1.1
9*	11.9	4.9
10	11.6	5.4
11	11.2	5.6

Patients 1 and 3 have achieved the protocol definition of transfusion independence†

*Male patients; ‡Hb supported by transfusions; †Weighted average Hb ≥ 9 g/dL without any RBC transfusions for ≥ 12 months; Hb, hemoglobin; VCN, vector copy number (vector copies/diploid genome)

Transfusion-free $Hb^{A\text{-}T87Q}$ and total Hb levels in blood are stable after LentiGlobin gene therapy

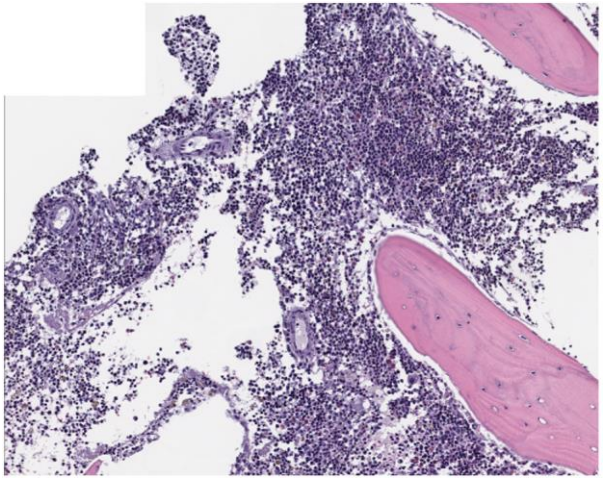


*Last Hb before patient restarted red blood cell transfusions; Hb, hemoglobin

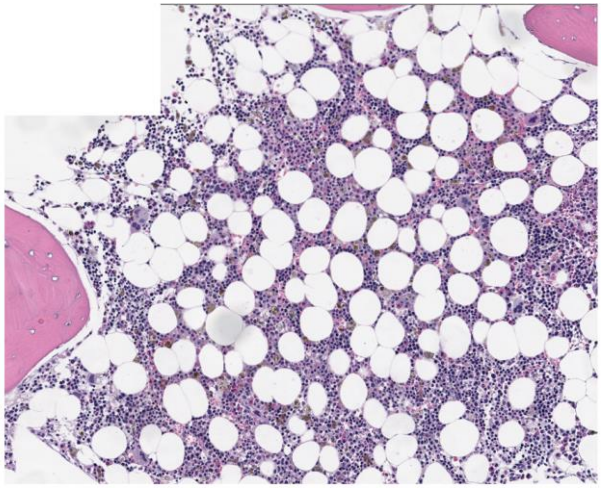
Improvement of erythropoiesis in a patient evaluated in HGB-207

Patient 1 (20 years old) bone marrow analysis

Screening

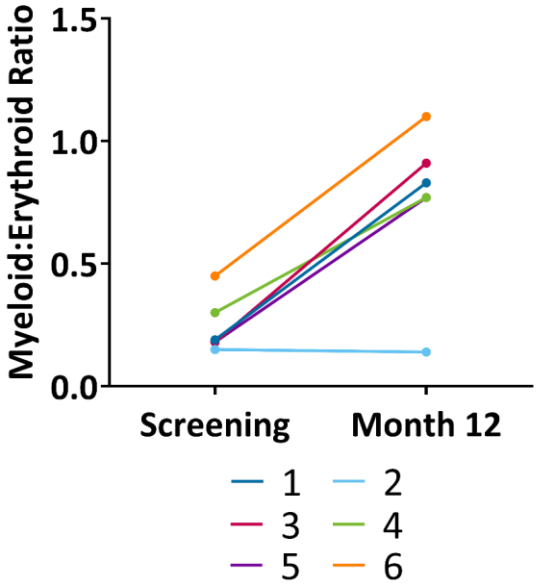


Month 12 post-LentiGlobin



Hb at Month 12: 13.1 g/dL

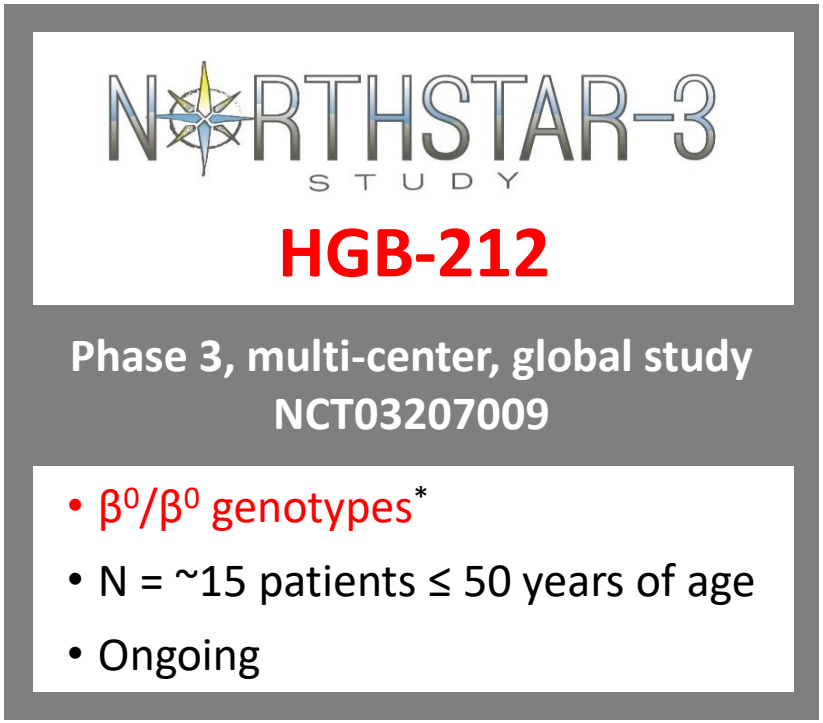
Myeloid / Erythroid ratio following LentiGlobin gene therapy



Normal M:E Ratio[1]: 3-4:1

Origa R. GeneReviews®. 2018.

Interim results of Phase III trial HGB-212



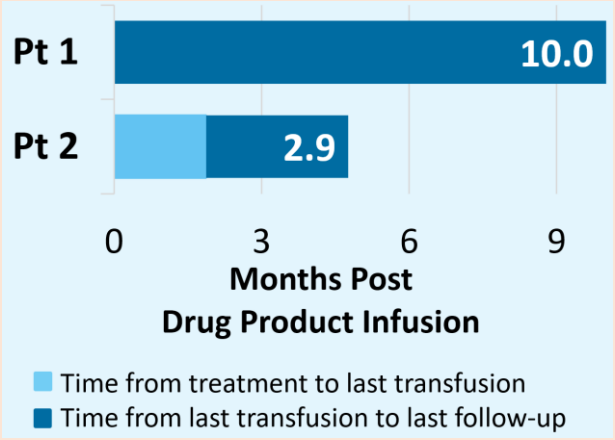
*Includes patients with the β^+ *HBB* mutation IVS I-110 (G→A)

Last data cut off = September 14, 2018

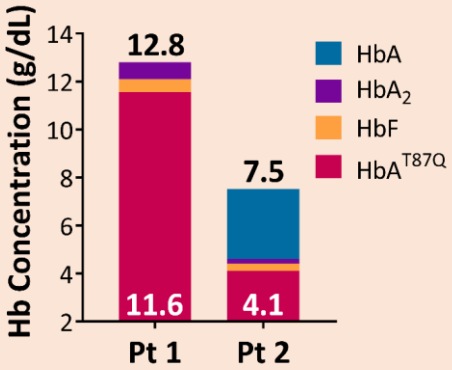
Drug Product Characteristics	N=3
Drug product cell dose CD34+ cells x10^6/kg, median (min – max)	**6.1** (5.9 – 12.9)
Drug product VCN[*] vector copies/diploid genome, median (min – max)	**3.3** (2.9 – 3.9)
CD34+ cells transduced[*] %, median (min – max)	**82** (78 – 85)
Treatment Characteristics	
Busulfan AUC[†] µM*min, median (min – max)	**5141** (4372 – 6351)
Neutrophil engraftment[‡] study day, median (min – max)	**34** (14 – 38)
Platelet engraftment[#] study day, N=2[^]	**28, 50**

Preliminary outcomes of patients (β^0/β^0) treated in HGB-212

Time free from transfusions in patients with ≥ 3 months follow-up



Hb fractions in patients with ≥ 3 months follow-up



	Pt 1	Pt 2
Peripheral VCN	3.4	2.2
Study Visit	M9	M3

Investigator-reported Hb at last visit**

	Hb (g/dL)	Last RBC transfusion
Pt 1 26 yr old male β^0/β^0	**13.8** M12	M0
Pt 2 7 yr old female β^0/β^+ (IVS-I-110)	**10.1** M6	M1.9
Pt 3 17 yr old male β^+/β^+ IVS-I-110 homozygous	**11.6** M3	M1.4

No serious AEs or DP-related AEs were reported following LentiGlobin infusion

*Includes investigator reported data as of November 19, 2018 not from programmed statistical outputs; †Hematologic AEs commonly observed post-transplant have been excluded.

**Data as of September 14, 2018 unless otherwise noted.

AEs, adverse events; DP, drug product; Hb, hemoglobin; RBC, red blood cell; VCN, vector copy number (vector copies/diploid genome).

Summary of clinical outcomes in β-Thalassemia

- **With follow-up extending to > 5 years, no serious adverse events* related to the vector has been detected** *(*one pending AE of thrombocytopenia possibly related to the drug product)*

- **The blood levels of $Hb^{A\text{-}T87Q}$ are similar across β-thalassemia genotypes**

- ***Refinements in LentiGlobin manufacturing from Phase I/II to Phase III:***
  HGB-204/205: 7 of the 22 patients express ≥ 6 g/dL of $Hb^{A\text{-}T87Q}$ by 6 months
  HGB-207: 7 of 8 patients express ≥ 7.6 g/dL of $Hb^{A\text{-}T87Q}$ by 6 months (up to 10.6 g/dL $Hb^{A\text{-}T87Q}$)

- **87.5% (21/24*) non-β^0/β^0-thalassemia subjects are free of transfusions in HGB-204/205/207 studies** *(*failures linked to low VCN)*, **with the longest follow-up > 5 years** *(Hb levels between 9.7 and 14.1 g/dL at last visit)*

- **54.5% (6/11*) $\beta^{0/0}$-thalassemia subjects are free of transfusions in HGB-204/205/212 studies** *(*incluant the first 2 patients in HGB-212)*
  or show a major decrease in transfusion requirements

- **Improvements in iron overload, metabolism and dyserythropoiesis**

Severe sickle cell disease

Proof of principle in Phase I/II trial HGB-205

The NEW ENGLAND JOURNAL of MEDICINE

March 2017

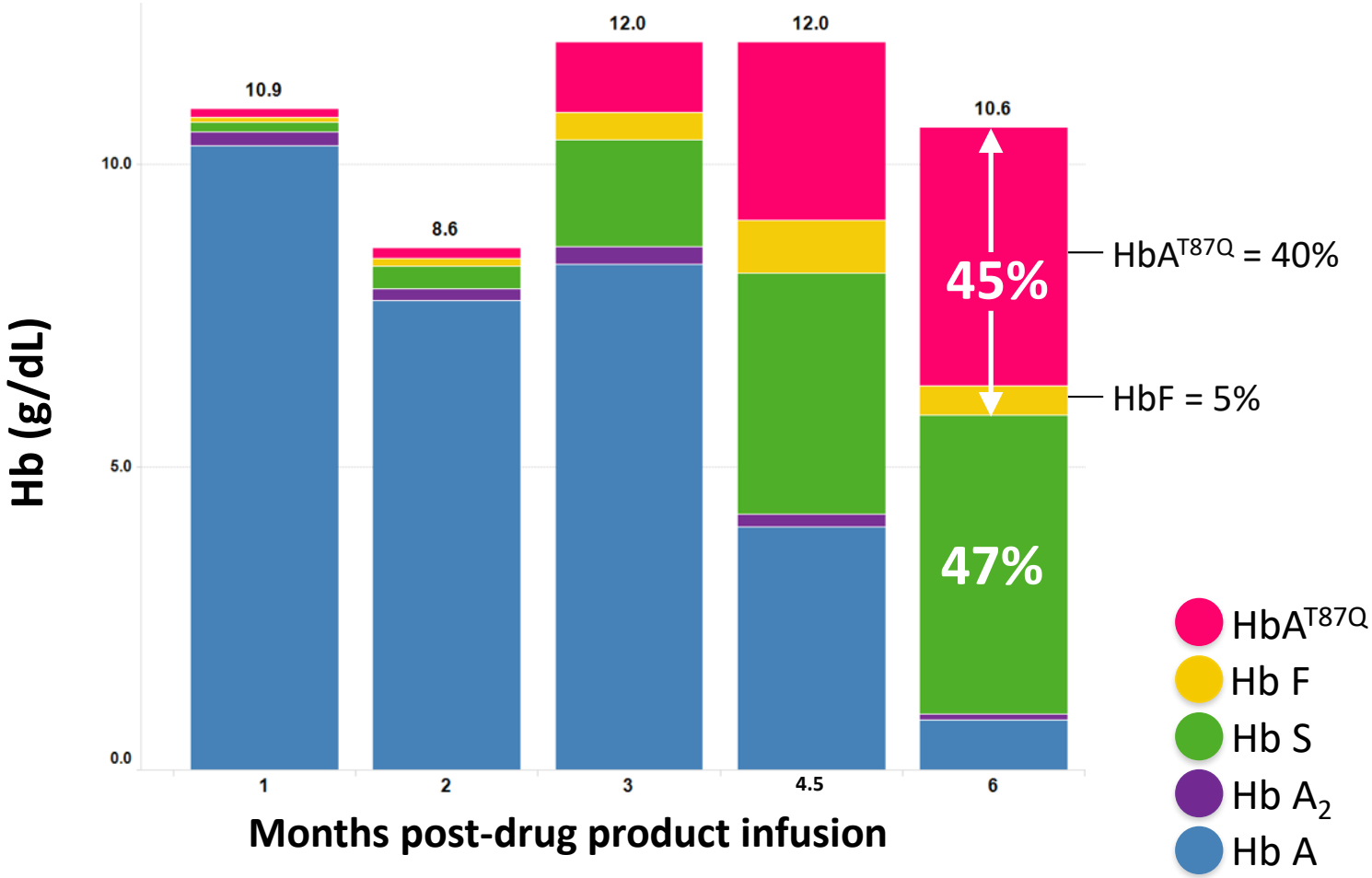
ORIGINAL ARTICLE

Gene Therapy in a Patient with Sickle Cell Disease

Jean-Antoine Ribeil, M.D., Ph.D., Salima Hacein-Bey-Abina, Pharm.D., Ph.D., Emmanuel Payen, Ph.D., Alessandra Magnani, M.D., Ph.D., Michaela Semeraro, M.D., Ph.D., Elisa Magrin, Ph.D., Laure Caccavelli, Ph.D., Benedicte Neven, M.D., Ph.D., Philippe Bourget, Pharm.D., Ph.D., Wassim El Nemer, Ph.D., Pablo Bartolucci, M.D., Ph.D., Leslie Weber, M.Sc., Hervé Puy, M.D., Ph.D., Jean-François Meritet, Ph.D., David Grevent, M.D., Yves Beuzard, M.D., Stany Chrétien, Ph.D., Thibaud Lefebvre, M.D., Robert W. Ross, M.D., Olivier Negre, Ph.D., Gabor Veres, Ph.D., Laura Sandler, M.P.H., Sandeep Soni, M.D., Mariane de Montalembert, M.D., Ph.D., Stéphane Blanche, M.D., Philippe Leboulch, M.D., and Marina Cavazzana, M.D., Ph.D.

N Engl J Med 2017; 376:848-855 | March 2, 2017 | DOI: 10.1056/NEJMoa1609677

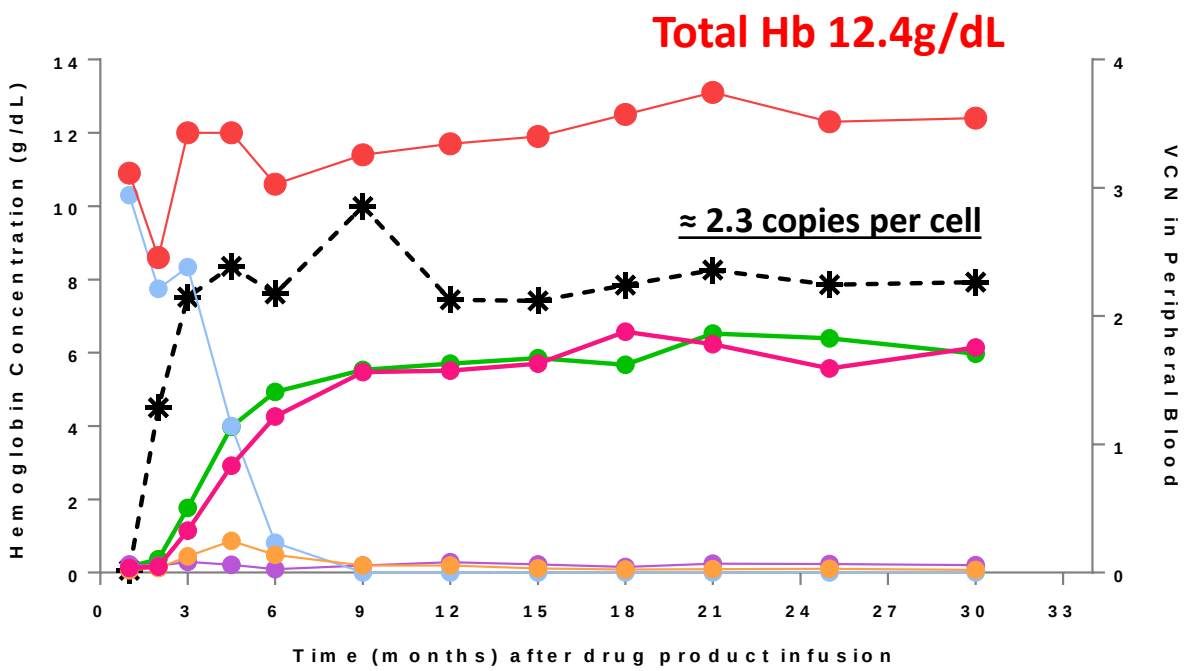
Rising levels of HbA^{T87Q} with endogenous anti-sickling HbF account for 45% of total Hb at Month 6, well above the 20-30% levels expected to be therapeutic



Subject 1204 (β^S/β^S) producing 4.3 g/dL HbA^{T87Q} (40%), 0.49 g/dL of HbF (5%) and 4.9 g/dL HbS (47%) at 6 months

Four years post gene therapy:

High, persistent level of integrated vector in peripheral blood leukocytes and durable HbA^{T87Q} expression



HbA^{T87Q} 50%

HbS 48%

HbA_2 2%

HbF <1%

(HbA 0%)

Ribeil *et al.* NEJM 2017; EHA 2017

Stable improvement of clinical outcomes and biological markers in first treated patient with SCD

Pre-Treatment

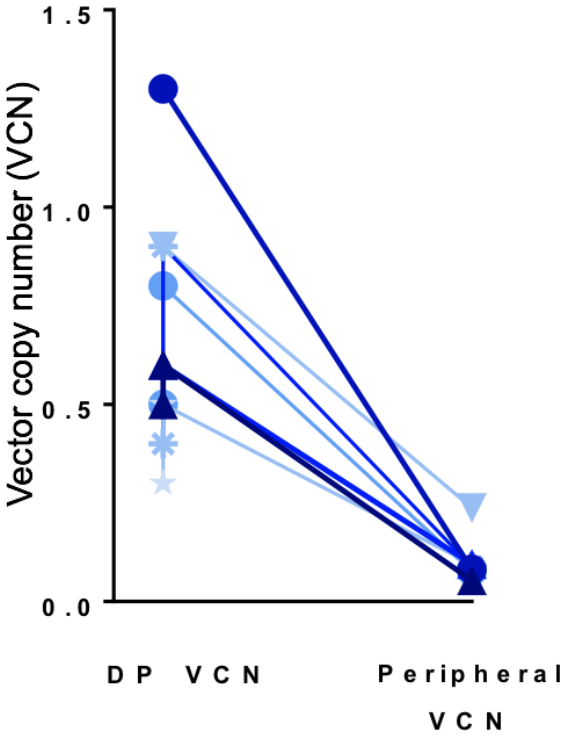
Transfusions	Monthly transfusions since 2010	**Weaned off transfusions** Last transfusion on Day + 88
Clinical Status	Multiple hospitalizations for painful VOCs and ACSbefore transfusion regimen	**Month +30:** a single VOC following a case of gastroenteritis leading to dehydration
Hemolysis	Baseline while on transfusion - Reticulocytes : 238 x 10^9/L - LDH: 626 U/L - Bilirubin : 50µM	**24 months after treatment** • **Reticulocytes 177 x 10^9/L** • **LDH 240 U/L** • **Bilirubin 15 µM**

Interim results of Phase I/II trial HGB-206

Last data cut off = September 14, 2018

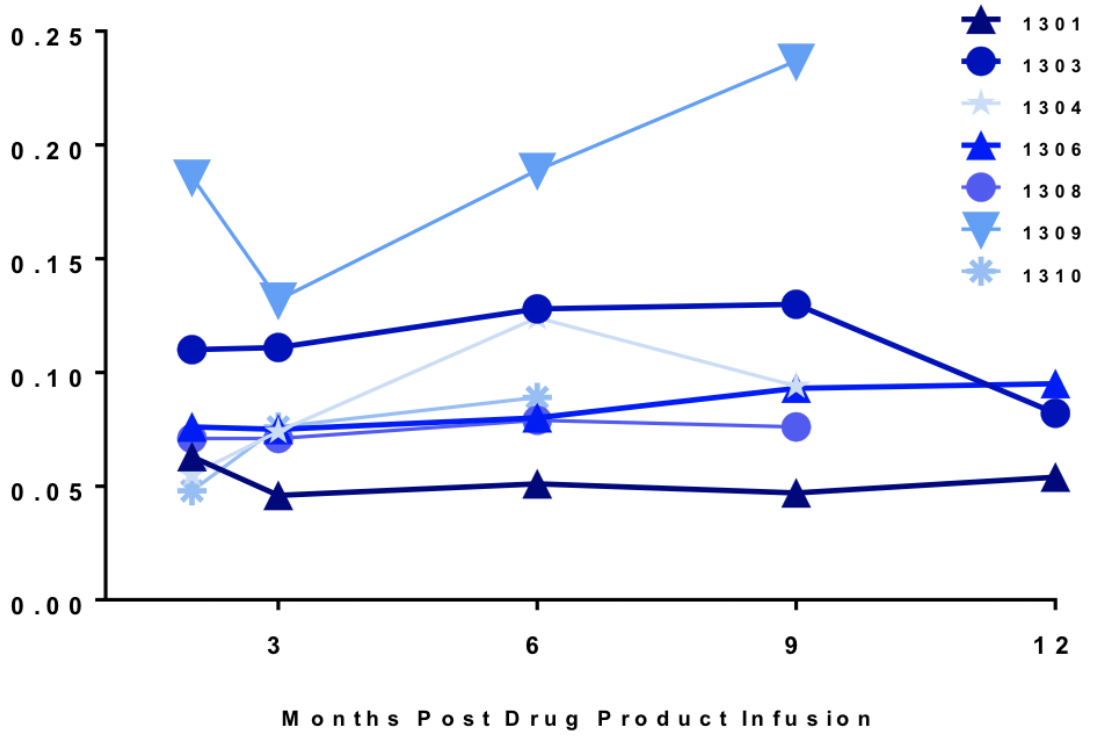
Disappointing initial results in the companion US trial (HGB-206) for SCD

VCN drop from drug product to peripheral blood



(at last measurement)
Median 0.09 (range 0.05 – 0.24)

Peripheral blood VCN over time

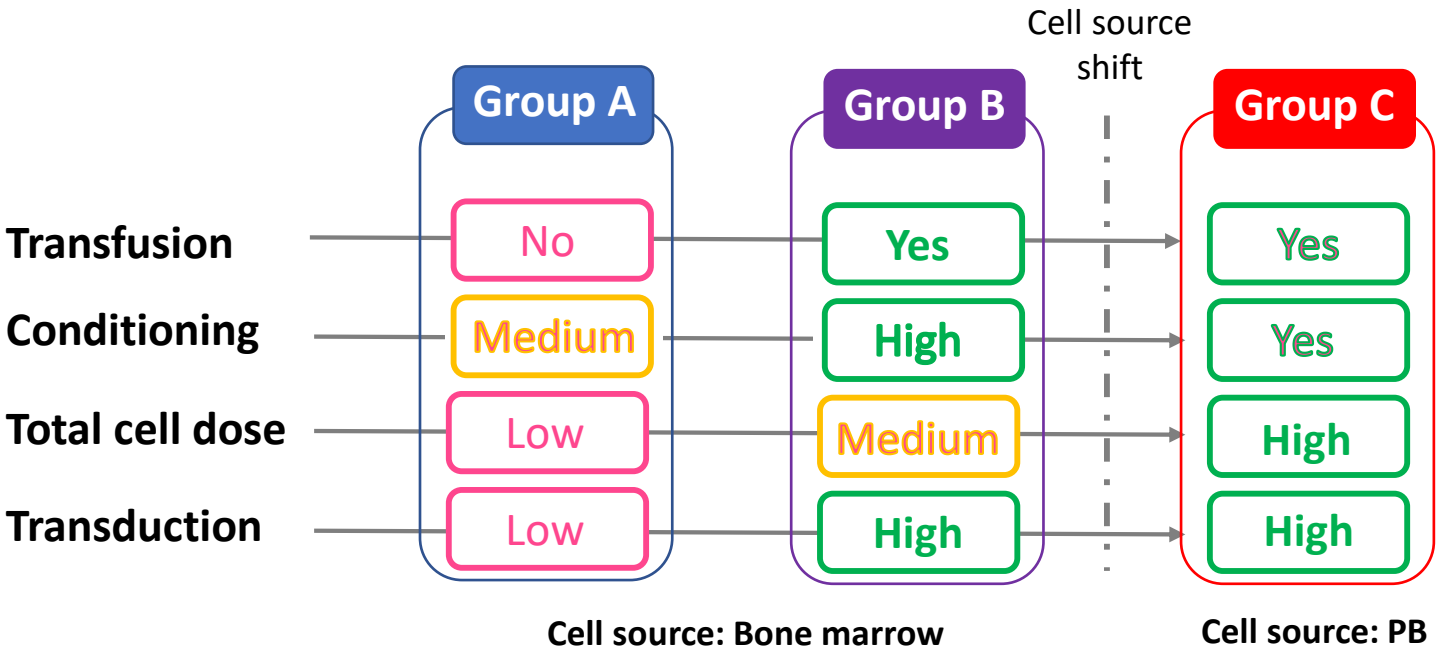


Kanter et al. 2016 ASH Meeting

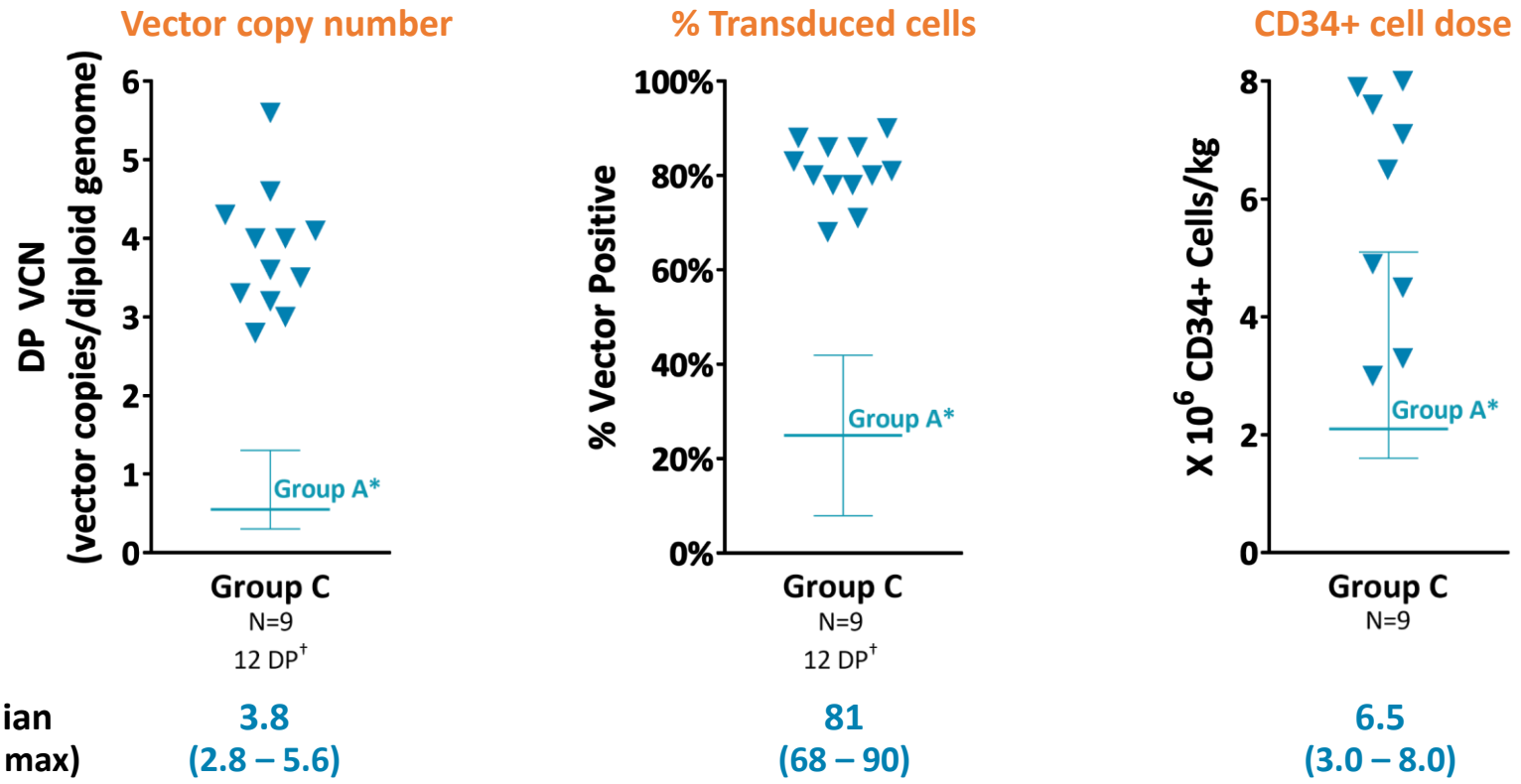
Evolution of HGB-206: Protocol and DP manufacturing changes

A number of parameters may explain the differences observed between HGB205 and HGB206 studies

- Conditioning
- Hypertransfusion (pre-treatment)
- CD34+ cell dose



HGB-206 Group C: Refinements to manufacturing and cell harvest led to improved drug product charateristics

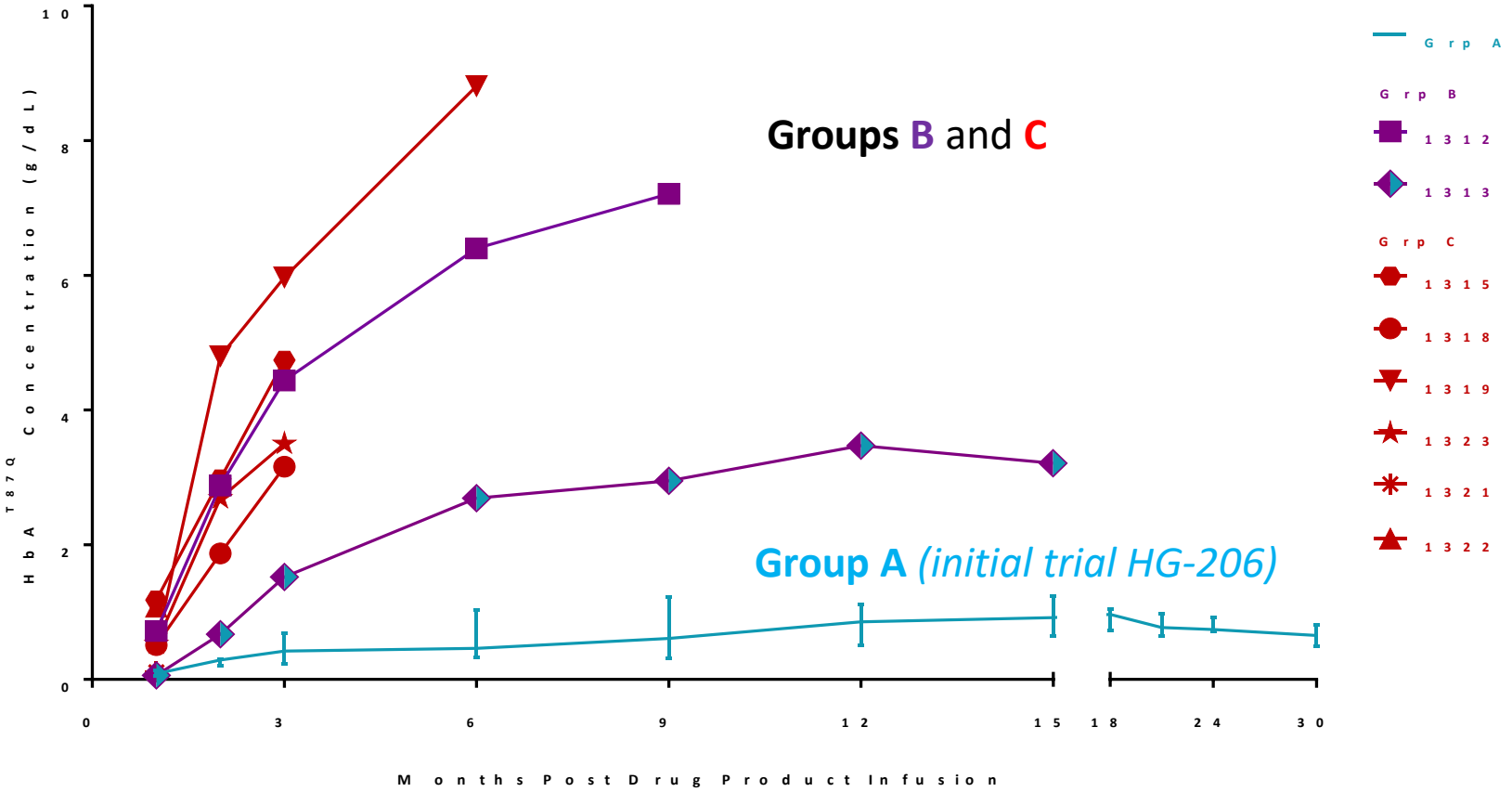


Median (min – max)	**3.8 (2.8 – 5.6)**	**81 (68 – 90)**	**6.5 (3.0 – 8.0)**

*Group A shown as median (min – max); †Number of DP exceeds number of patients since some patients were harvested or mobilized more than once

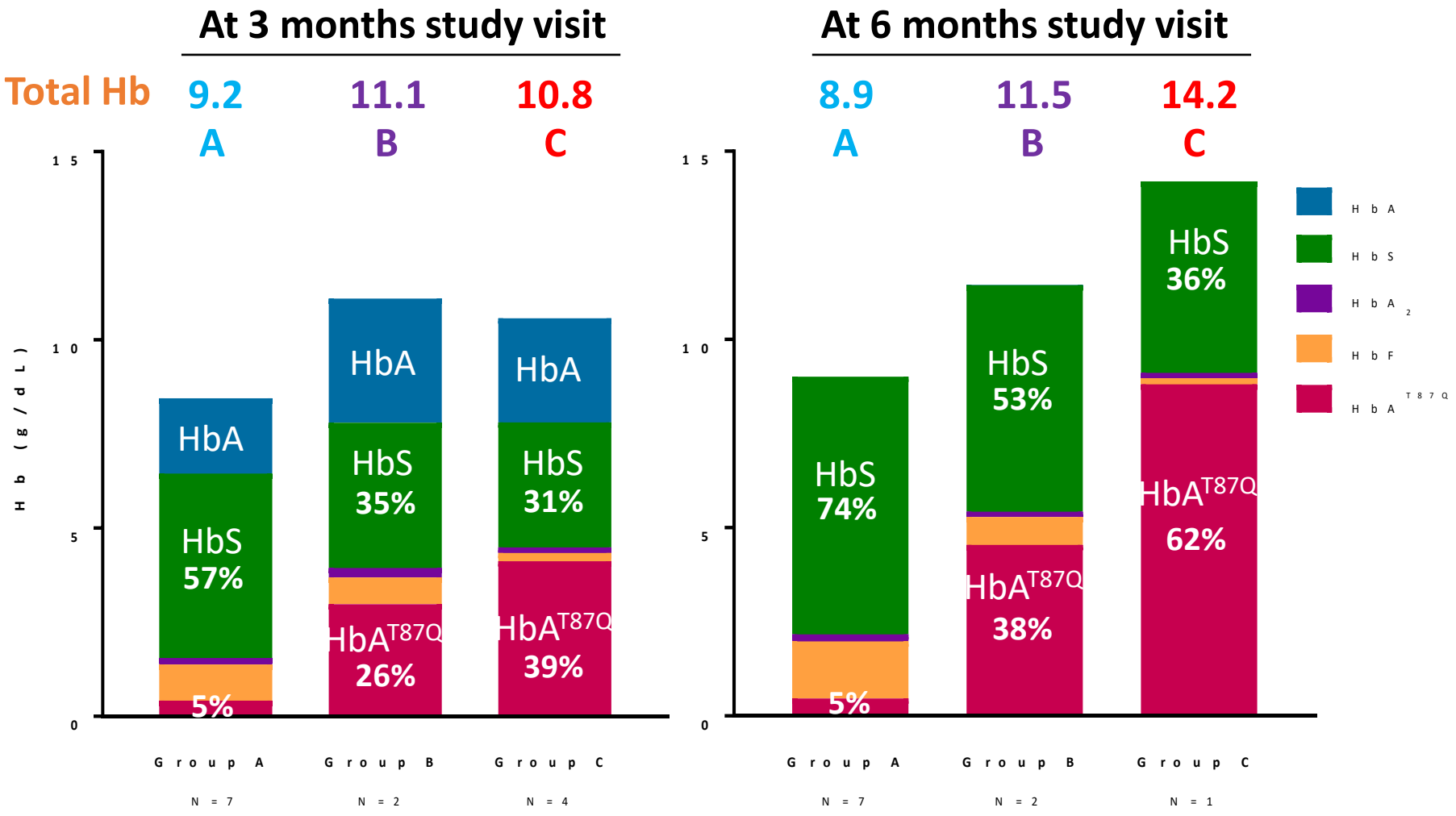
DP, drug product; VCN, vector copy number

Patients in Group B and C show much improvement in $Hb^{A\text{-}T87Q}$ production



Data collected in May 2018

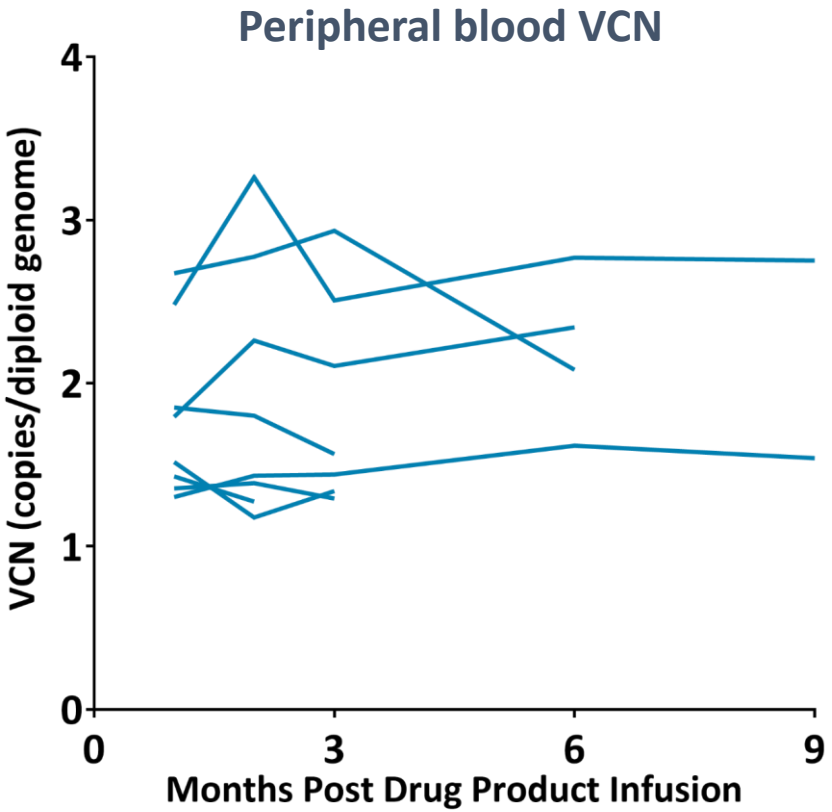
Vector derived blood Hb levels at 3 and 6 months after gene therapy

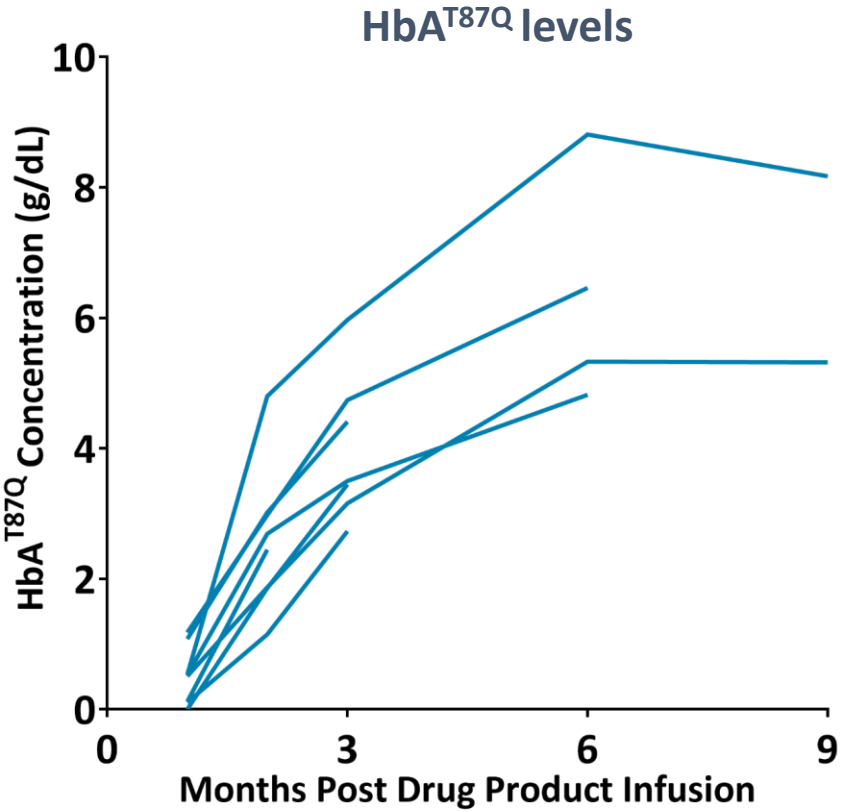


% represent median Hb fractions as % of total, except for Group C at 6 months given N=1

Data collected in May 2018

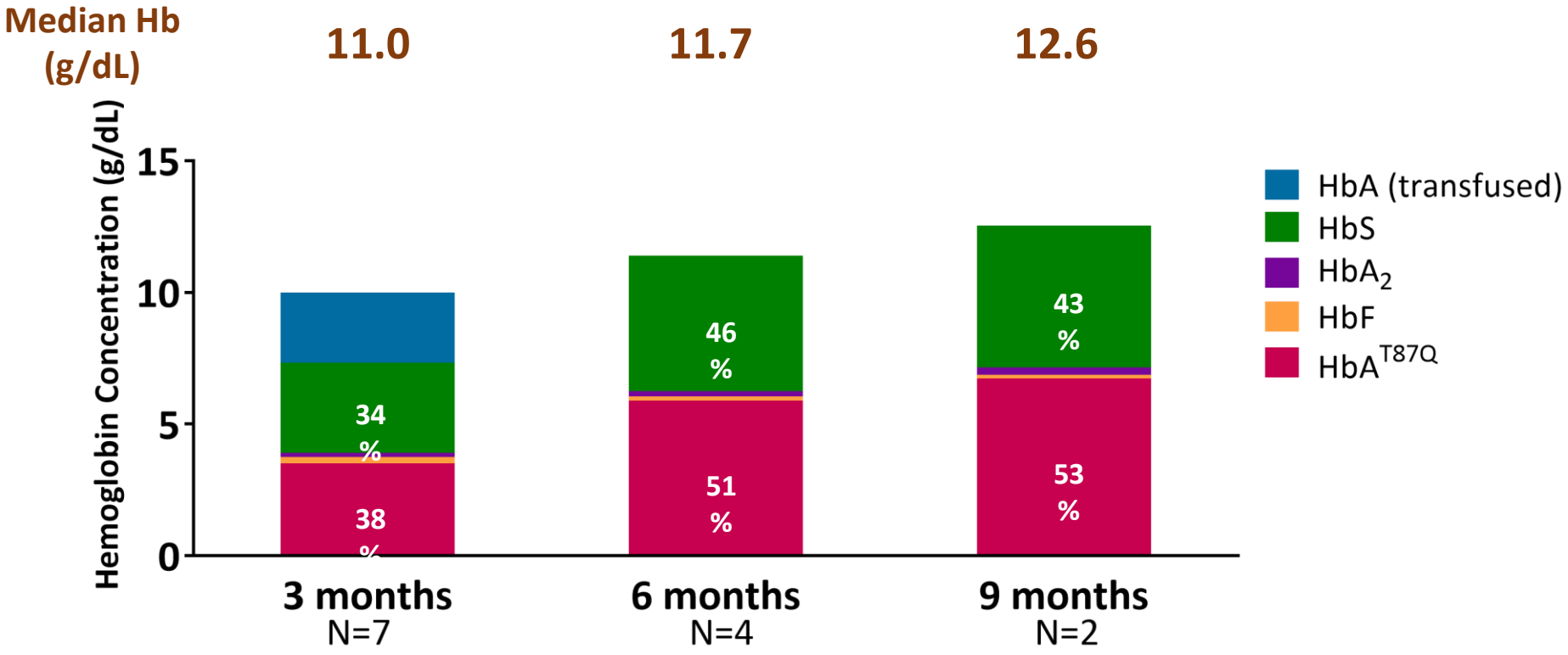
HGB-206 Group C: Peripheral blood VCN and $Hb^{A\text{-}787Q}$ over time





Hb, hemoglobin; VCN, vector copy number

HGB-206 Group C: LentiGlobin derived $Hb^{A\text{-}T87Q}$ equals or exceeds HbS levels at > 6 months

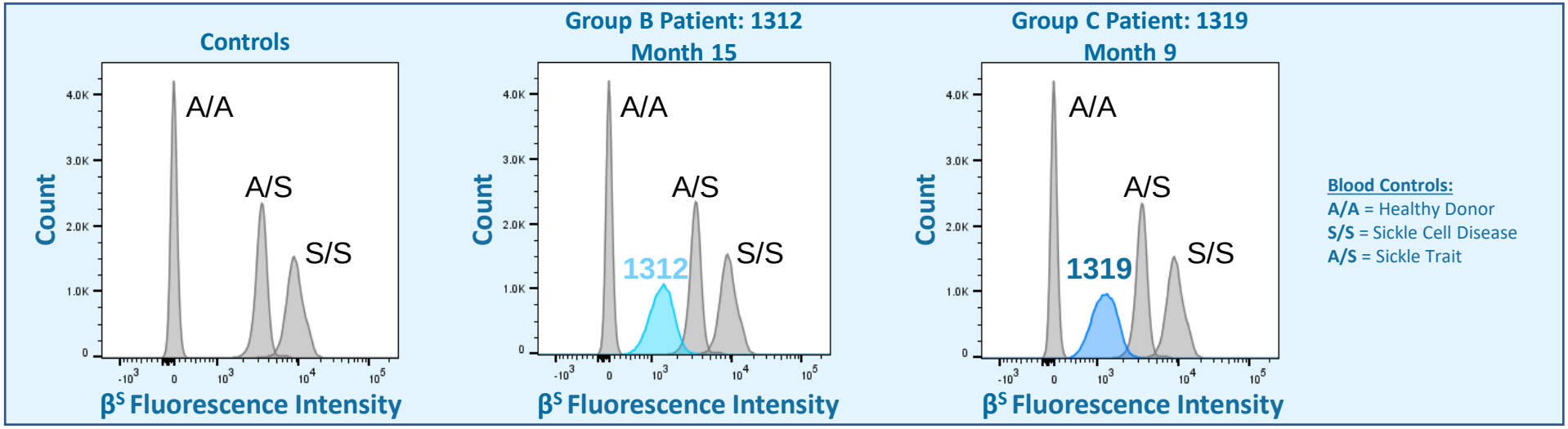


% represent median Hb fractions as % of total

Hb, hemoglobin

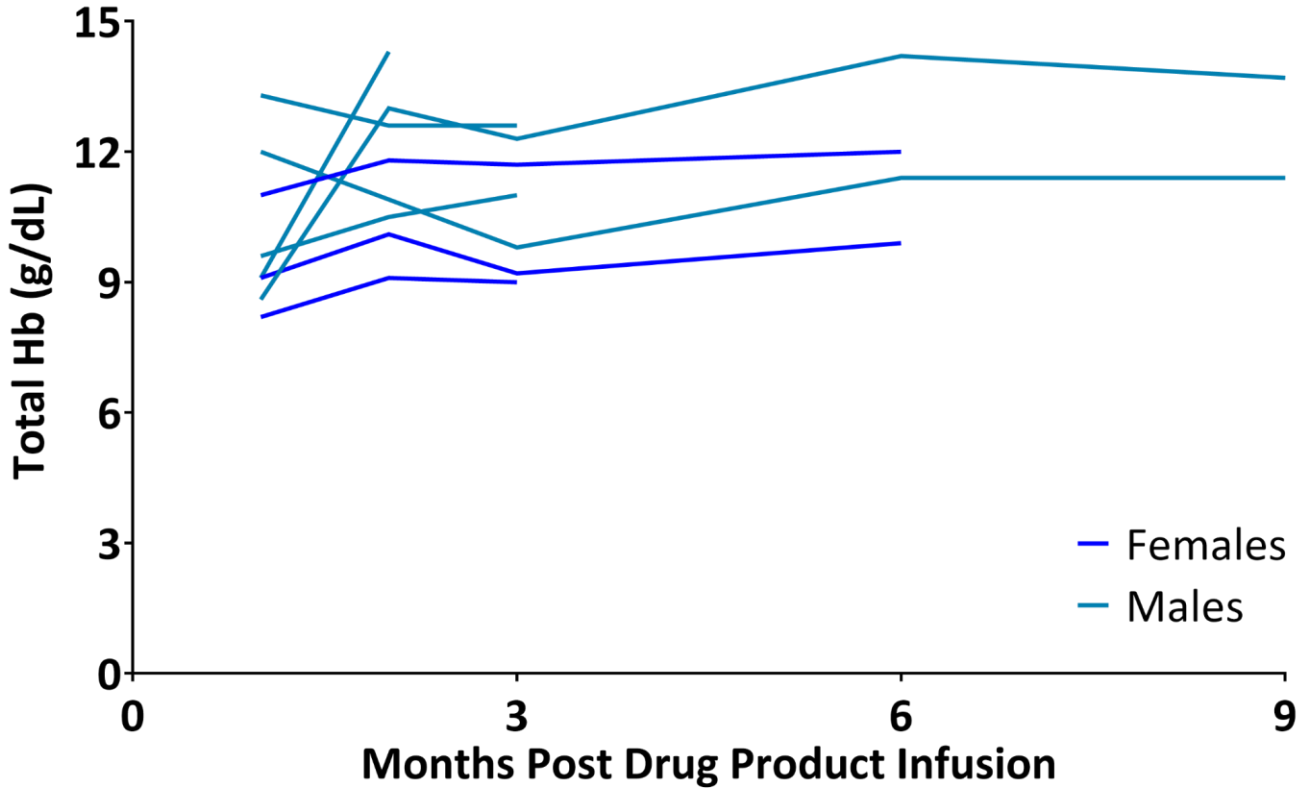
Intracellular RBC staining with anti-β^S antibody suggests pancellular distribution of LentiGlobin-derived HbA^{T87Q} is achievable

- Exploratory assay: using an antibody that recognizes β^S, performed intracellular staining of RBCs followed by FACS analysis
  - Fluorescence intensity (X-axis) indicates amount of β^S in cells in sample
  - Control A/A, A/S, and S/S samples showed clearly distinct β^S intensity distributions, with S/S > A/S > A/A
- **Initial results in 2 patients 9 and 15 months post treatment show that nearly all RBCs have lower β^S intensity than S/S, and even A/S, samples**
  - Most non-β^S globin in these samples is $\beta^{A\text{-}T87Q}$ – patients are off transfusions and HbF < 2.5% of total globin chains



FACS, fluorescence-activated cell sorting; RBCs, red blood cells

HGB-206 Group C: Stable unsupported Hb levels over 3-9 months follow-up



Hb, hemoglobin

Updated interim summary for HGB -206 Group C Phase I/II study

- LentiGlobin gene therapy in patients with severe SCD demonstrates an acceptable safety profile
- Refined manufacturing using plerixafor-mobilized HSCs generates robust HbA^{T87Q} production of 4.8 – 8.8 g/dL at ≥ 6 months that equals or exceeds HbS levels
  - Total unsupported Hb of 9.9 – 13.7 g/dL at last visit
  - Decreased hemolysis following LentiGlobin gene therapy
- No VOEs observed in any Group C patient following LentiGlobin treatment
- Data further support safety and feasibility of plerixafor mobilization and apheresis in SCD
- Exploratory translational assay suggests pancellular expression of gene therapy-derived Hb
- Protocol amended with expanded enrollment and modified endpoints to further evaluate the clinical impact of LentiGlobin gene therapy in SCD

Hb, hemoglobin; HSC, hematopoietic stem cell; VOE, vaso-occlusive event

- On the basis of results from said trials, the European regulatory agency **EMEA has granted accelerated review status** of *bluebird bio*'s application for BB305 product market. **Request for market approval of BB305 has been filed by *bluebird bio* for non-β^0 genotypes (e.g., β^E/β^0) in October 2018. EMEA has 150 days to issue a decision.**

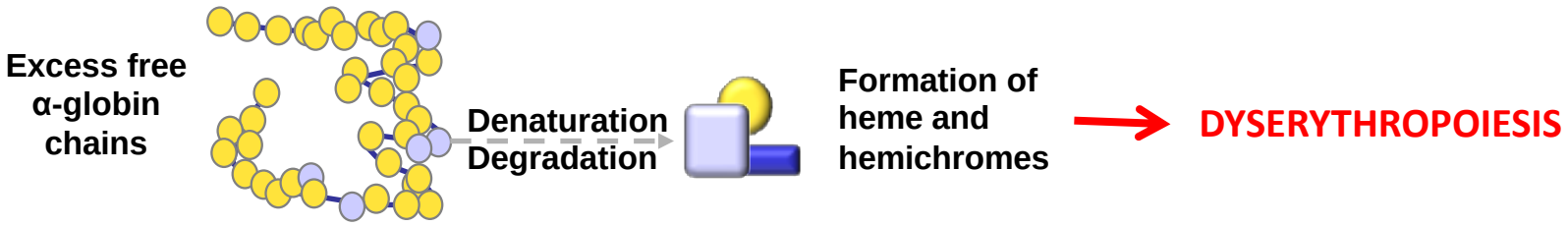
  Follow-up filing in the US is planned with FDA.

- A similar clinical development program for Sickle Cell Disease with the current BB305 vector is underway.

For β^0-Thalassemia, we feel there is room for vector/protocol improvement. Our goal is to achieve complete and sustained disease correction without the need to increase the mean vector copy number (VCN) further.

α-Thalassemia is a known key modifier of β-Thalassemia

- In β-thalassemia, unbound α-globin forms toxic aggregates in developing RBCs, resulting in apoptosis of erythroid progenitors (dyserythropoiesis) and decreased RBC lifespan.



- Natural co-inheritance of α-thalassemia with β-thalassemia results in a much less severe condition, due to reduction of excess α-globin and normalization of α:β globin ratio.

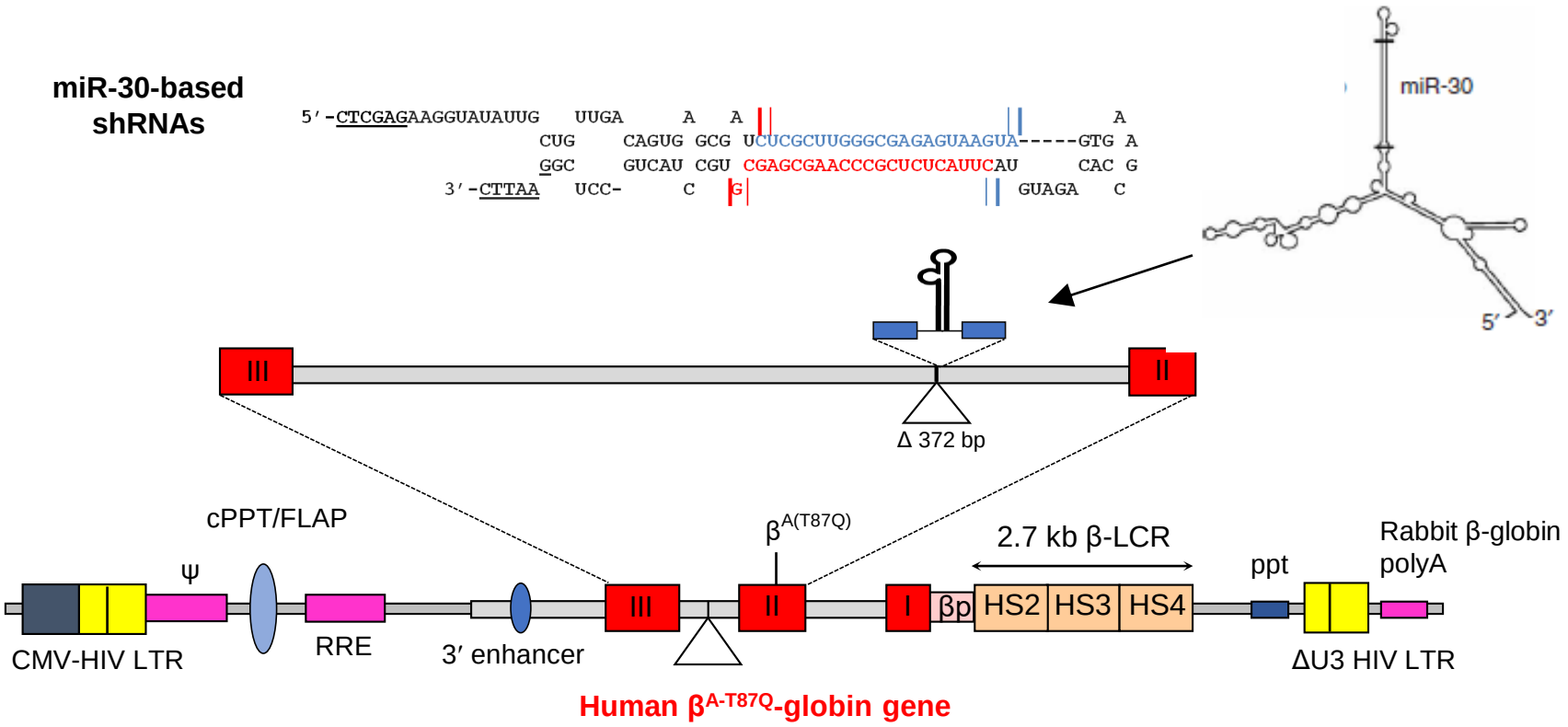
Review Article 2015

α-Globin as a molecular target in the treatment of β-thalassemia

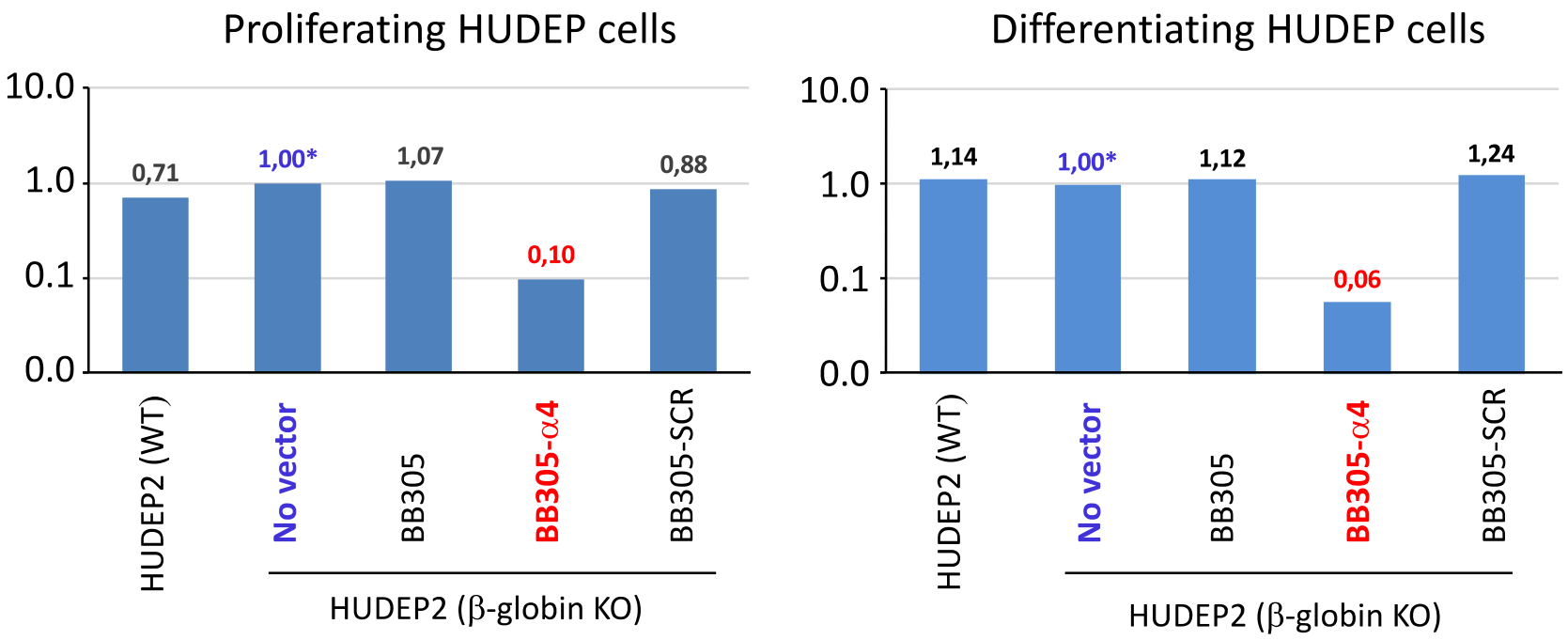
Sachith Mettananda,[1,2] Richard J. Gibbons,[1] and Douglas R. Higgs[1,3]

Co-expressing $\beta^{A\text{-}T87Q}$-globin and an intronic microRNA against human α2-globin within BB305

- shRNA is incorporated into a well-characterized mir30 scaffold.
- mir30 shRNA inserted into human $\beta^{A\text{-}T87Q}$ IVS 2 of BB305 at the pre-existing deletion breakpoint.
- Production of shRNA is thus linked to erythroid expression of $\beta^{A\text{-}T87Q}$ -globin.



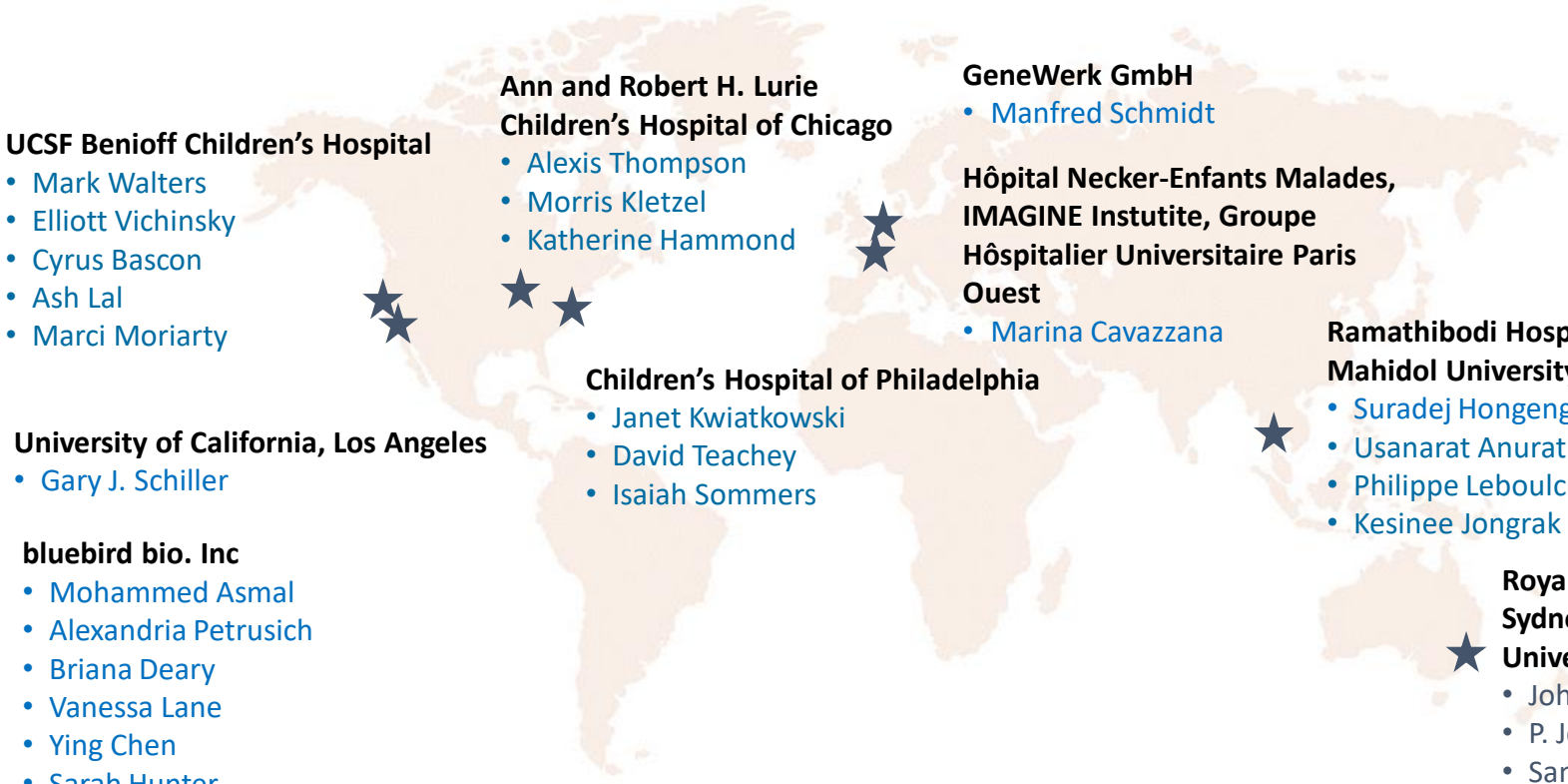
human α2 / α1-globin mRNA ratios decrease by 90% in HUDEP cells (with KO of human β-globin) after transduction with BB305-shα4



** Normal α2 / α1-globin mRNA ratios are ~2.5. Data have been normalized for this ratio in the « no vector » control (value =1).*

HGB-204 and HGB-205

Thank you to the study participants and their families



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- Katherine Hammond

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- Kesinee Jongrak

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HGB-207 and HGB-212

Thank you to the study participants and their families

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- Kimberly Price
- Marisa Gayron
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- Srujana Sarikonda

HGB-206

Thank you to the study participants and their families

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- Monica Bhatia

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- Sarah Hunter
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- Liz Macari
- Calvin Lee
- Purvi Mody
- Iva Kronja

“Anti-alpha Project”

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