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LentiGlobin Gene Therapy in Patients with Sickle Cell Disease: Updated Interim Results from HGB-206

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HGB-206: Study of LentiGlobin gene therapy for severe sickle cell disease (SCD)

HGB-206

Key Enrollment Criteria

- 18+ years of age
- History of symptomatic SCD
- Adequate organ function
- No previous HSCT or gene therapy

Target enrollment: up to 29

Study Objectives

- Primary objective: Safety
- Key secondary objectives:
  - Frequency of VOCs and ACS
  - Vector copy number in peripheral blood
  - HbA^{T87Q} production, Hb, Hb fractions

Study initiated August 2014

ACS, acute chest syndrome; Hb, haemoglobin; HSCT, haematopoietic stem cell transplant; VOC, vaso-occlusive crisis

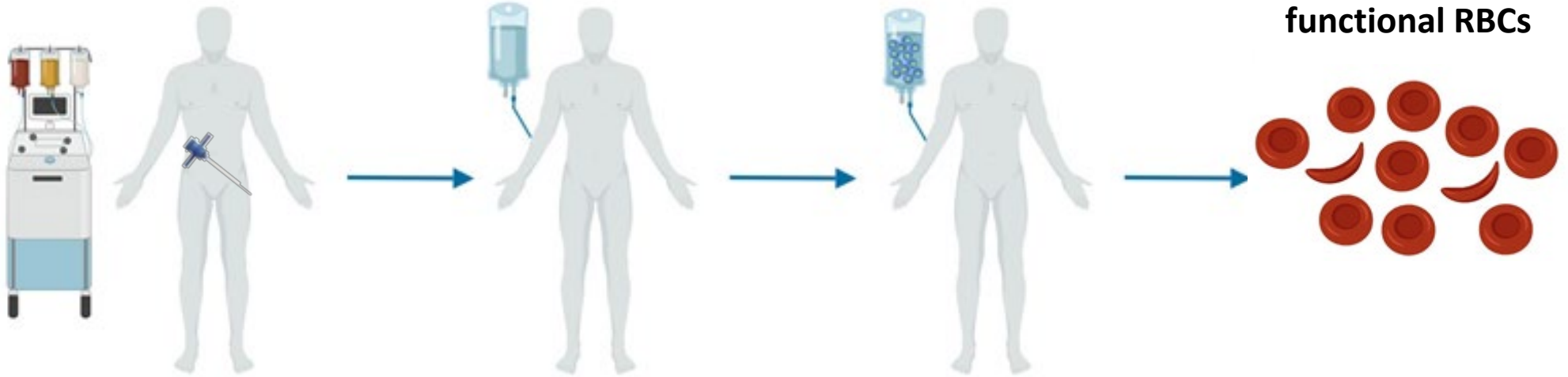
HGB-206: LentiGlobin gene therapy overview in patients with SCD

HSC collection
Bone marrow harvest or mobilisation with plerixafor & apheresis

Busulfan myeloablative conditioning

DP infusion

Transduced HSCs engraft and contribute to reconstitution of functional RBCs



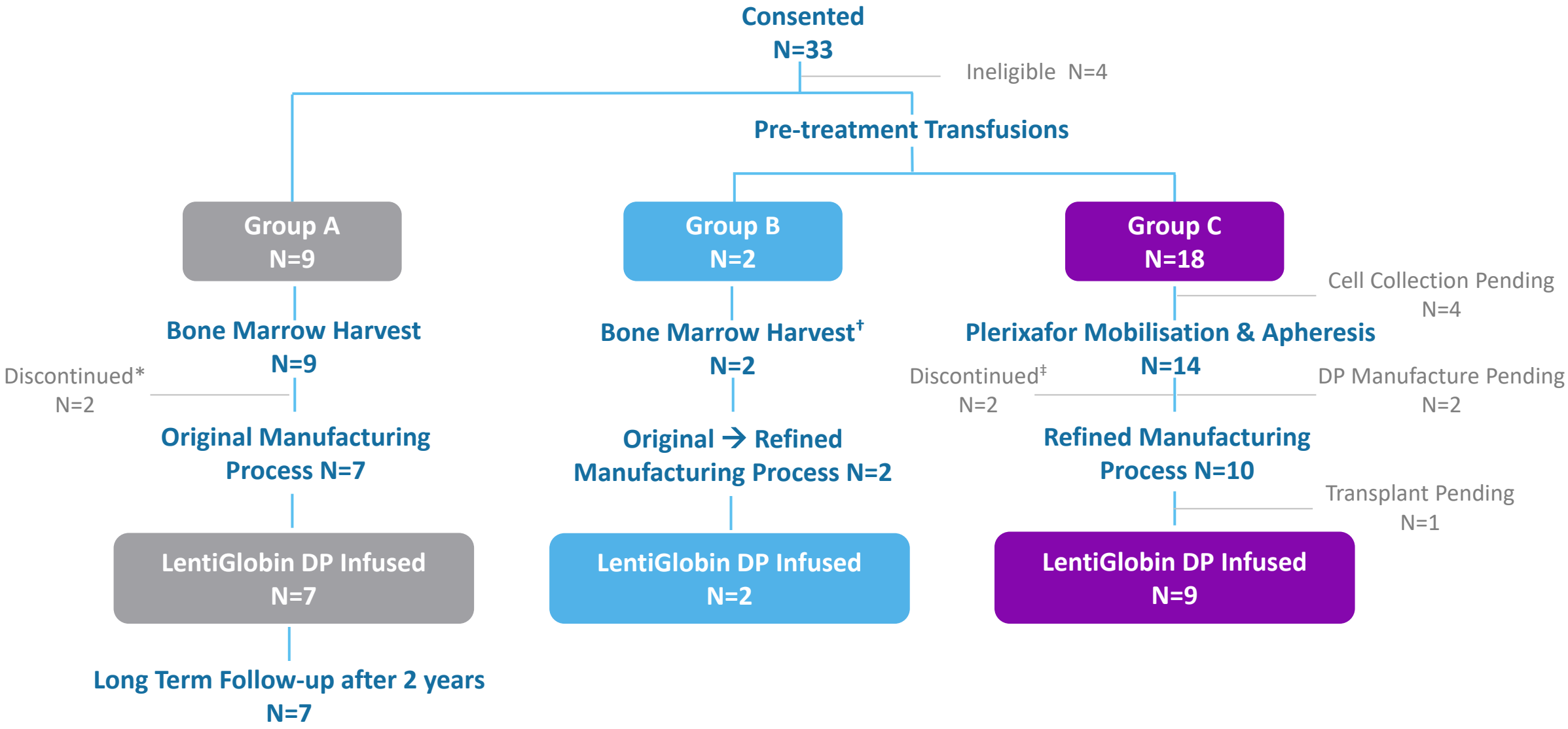
2-yr follow-up

Long-Term Follow-Up Study

	Group A	Group B	Group C
Pre-collection transfusion regimen	Optional	Required	Required
HSC source	Bone marrow	Bone marrow	Mobilised PB
Manufacturing process	Original	Orig → Refined	Refined

DP, drug product; HSC, haematopoietic stem cell; PB, peripheral blood; RBCs, red blood cells

HGB-206: Study disposition



*1 due to insufficient cell collection, 1 withdrew consent; †1 patient also received a single mobilisation cycle to collect cells for back-up; ‡1 due to adverse event, 1 withdrew consent

HGB-206: Patient characteristics

N=25 patients who started cell collection

Parameter	Group A N=9	Group B N=2	Group C N=14
Age at consent, years median (min – max)	**26** (18 – 43)	**24.5** (22 – 27)	**25.5** (18 – 36)
Gender	**2 F 7 M**	**0 F 2 M**	**6 F 8 M**
Genotype, β^S/β^S	**9**	**2**	**14**
SCD History			
Hydroxyurea, n	**5**	**2**	**8**
VOCs[*], n Annualized no. of events, median (min – max)	**7** **4.5** (2.0 – 27.5)	**2** **10.0** (2.5 – 17.5)	**9** **6.5** (3.5 – 14.0)
ACS[†], n Annualized no. of events, median (min – max)	**1** **1**	**1** **1**	**2** **1** (1 – 1)
Stroke, n	**2**	**0**	**3**
TRJV >2.5 m/s, n	**1**	**0**	**0**

*≥2 events/year in preceding 2 years; [†]≥2 episodes in preceding 2 years, with ≥1 episode in the past year or in the year prior to the initiation of regular transfusions

ACS, acute chest syndrome; F, female; M, male; TRJV, tricuspid regurgitant jet velocity; VOC, vaso-occlusive crisis

HGB-206: Treatment characteristics

N=18 infused patients

Parameter	Group A N=7 Median (min – max)	Group B N=2 (Pt 1312, Pt 1313)	Group C N=9 Median (min – max)
No. of bone marrow harvests	**2** (1 – 4)	**2, 3**	N/A
No. of mobilisation cycles	N/A	1[†]	**2** (1 – 3)
No. of apheresis procedures per mobilisation cycle	N/A	1	**1** (1 – 2)
CD34+ cells collected per collection cycle, x10^6 cells/kg	**4.0** (0.1 – 10.8)	**6.3**[‡]**, 1.2**[‡]	**9.2** (5.6 – 21.6)
Average busulfan AUC, µM*min (over 4 days)	**4747** (4084 – 5290)[#]	**5256, 5017**	**4787** (4608 – 5182)
Follow-up, months	**29.9** (29.2 – 38.9)	**14.3, 17.2**	**5.2** (0.5 – 9.2)
Neutrophil engraftment, days (ANC ≥ 500 /µl)	**22** (17 – 29)	**23, 28**	**19.5** (18 – 24)[§]
Platelet engraftment, days (platelets > 50k /µl)	**56** (29 – 63)	**31, 61**	**28** (19 – 136)[^]

[†]For research purposes; [‡]Median per BMH; [#]Based on data for 6 patients; [§]Based on data for 8 patients; [^]Based on data for 7 patients

- 6/7 Group C patients had platelet engraftment by Day 90

HGB-206: Safety profile with plerixafor mobilisation/apheresis vs bone marrow harvest (BMH)

In 26 BMHs in 11 patients, 18 Grade ≥ 3 AEs were reported in 6 patients*

Patients with Grade ≥ 3 AEs within 7 days of BMH	n (%) N=11
Procedural pain[1]	6 (54)
Anaemia	2 (18)
Vaso-occlusive pain[2]	2 (18)
Lymphocyte count increased	1 (9)

[1]Considered serious in 2 patients; [2]3 events in 2 patients, all considered serious

In 35 apheresis procedures in 14 Group C patients†, 5 Grade ≥ 3 AEs were reported in 3 patients

Patients with Grade ≥ 3 AEs within 7 days of 1st plerixafor in a mobilisation cycle	n (%) N=14
Vaso-occlusive pain[1]	2 (14)
Abdominal pain	1 (7)
Hypomagnesaemia	1 (7)
Non-cardiac chest pain	1 (7)

[1]Were considered serious and consistent with patients' histories of VOEs

*Patient could have experienced same AE more than once; †Plerixafor mobilisation/aphaeresis for research purposes was performed in 1 Group B patient, resulting in 3 Grade 3 AEs: vaso-occlusive pain, increased AST and increased ALT

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; VOE, vaso-occlusive event

HGB-206: Safety profile consistent with myeloablative busulfan conditioning

Non-haematologic Grade ≥ 3 AEs* *Post DP infusion in ≥ 2 patients*	n (%) N=18
Stomatitis	13 (72)
Febrile neutropaenia	11 (61)
Vaso-occlusive pain	5 (28)
Pharyngeal inflammation	4 (22)
Bacteraemia	2 (11)
Dyspnoea	2 (11)
Epistaxis	2 (11)
Non-cardiac chest pain	2 (11)
Pyrexia	2 (11)

- No cases of VOD
- No VOEs post DP infusion in Group C patients
- No graft failure, deaths or vector-mediated RCL
- No evidence of clonal dominance observed to date
- Serious AEs were reported in 12 patients, vaso-occlusive pain was most common (n=5; 4 in Group A & 1 in Group B)
  - **1 Grade 4 SAE of myelodysplastic syndrome (MDS) in Group A patient ~36 months post DP infusion**

*Haematologic AEs commonly observed post-transplant have been excluded

AE, adverse event; DP, drug product; RCL, replication competent lentivirus; SAE, serious adverse event; VOD, veno-occlusive liver disease; VOE, vaso-occlusive event

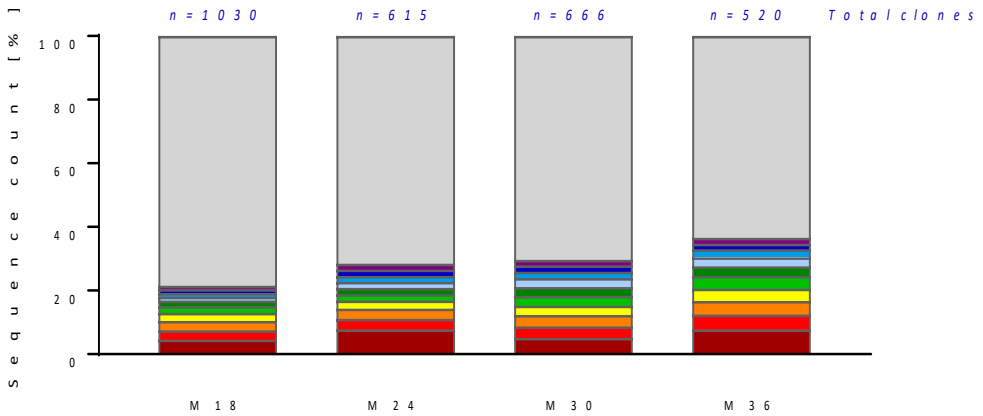
HGB-206: A case of myelodysplastic syndrome with excess blasts in a patient >40 years old

- **Treated with hydroxyurea for 8 years before enrollment; restarted post LentiGlobin DP infusion**
- **Received 3.3 mg/kg (200 mg) daily IV busulfan conditioning over 4 days**
- **BM biopsy showed 15% myeloblasts/dysplasia with monosomy 7 and 19p abnormality in 8/20 metaphases**

No evidence of clonal dominance (No IS > 30%)

- Largest 5 clones varied over 18 months

Frequencies of top 10 integration sites



Blast cells (CD34+) had low VCN consistent with no LVV genotoxicity

Marrow cell populations after MDS diagnosis	Purity (%)	VCN (c/dg)
Unsorted	N/A	0.14
CD34-	98	0.21
CD34+ blasts	93	0.02

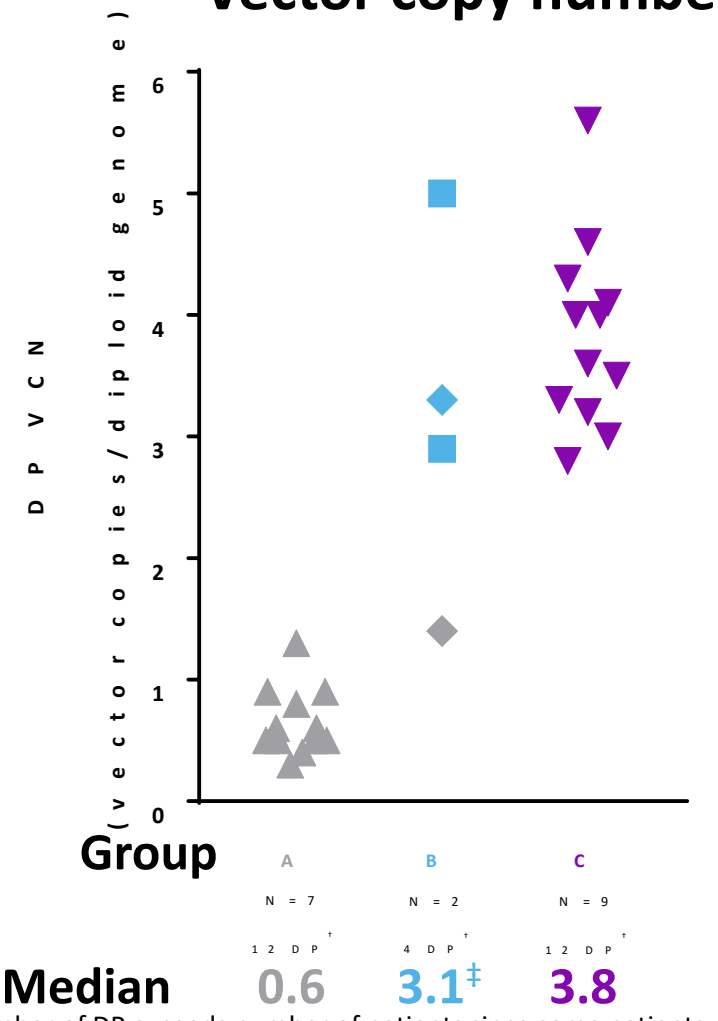
- Given low VCN in CD34+ blasts, MDS SAE is considered unlikely related to LentiGlobin*
- MDS is a risk of autologous HSCT with alkylating agents such as busulfan[1-3]

*Per safety database; BM, bone marrow; c/dg, copies/diploid genome; GT, gene therapy; HSCT, haematopoietic stem cell transplant; IS, integration site; LVV, lentiviral vector; MDS, myelodysplastic syndrome; N/A, not applicable; VCN, vector copy number

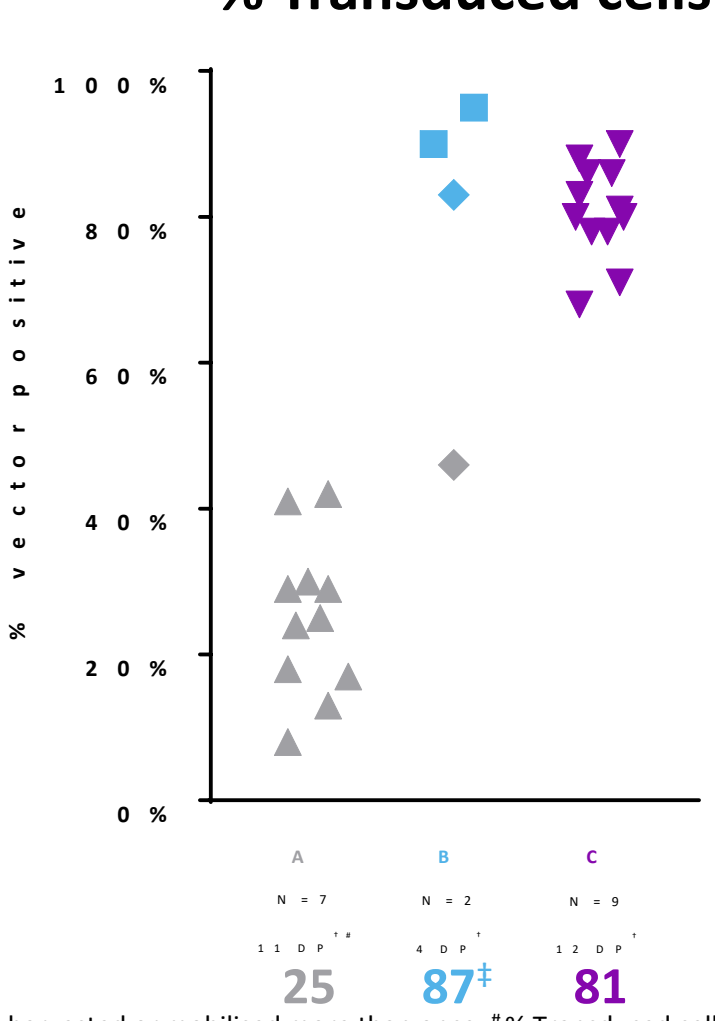
1. Rege KP et al., BMT 1998; 2. Howe R et al., BMT 2003; 3. McNerney ME et al., Nat Rev Cancer 2017

HGB-206: Refinements to manufacturing and cell harvest improved drug product characteristics

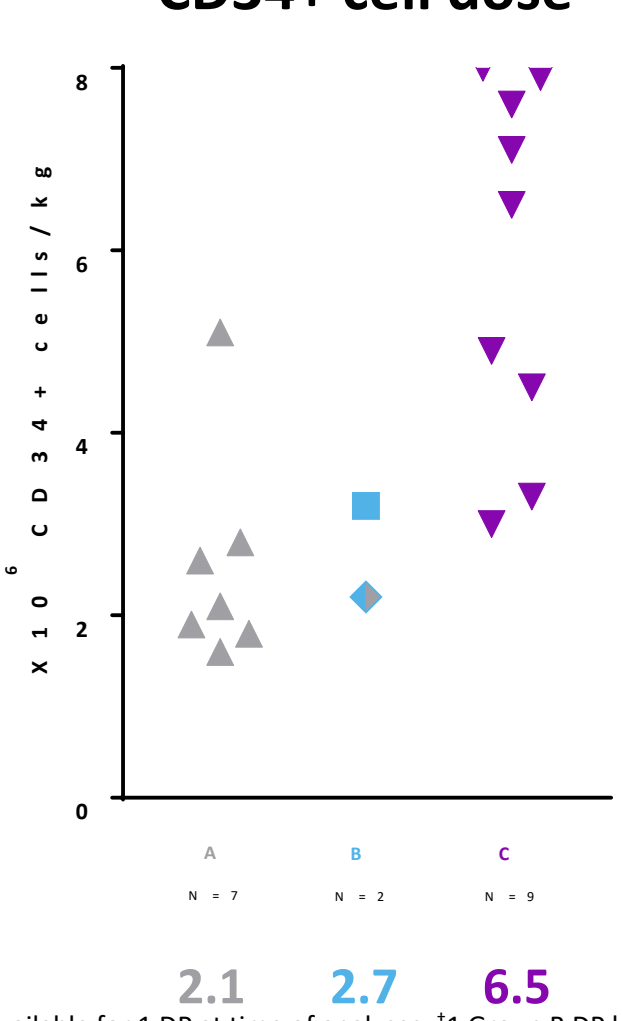
Vector copy number



% Transduced cells



CD34+ cell dose



LentiGlobin manufacturing process
(HSC source)

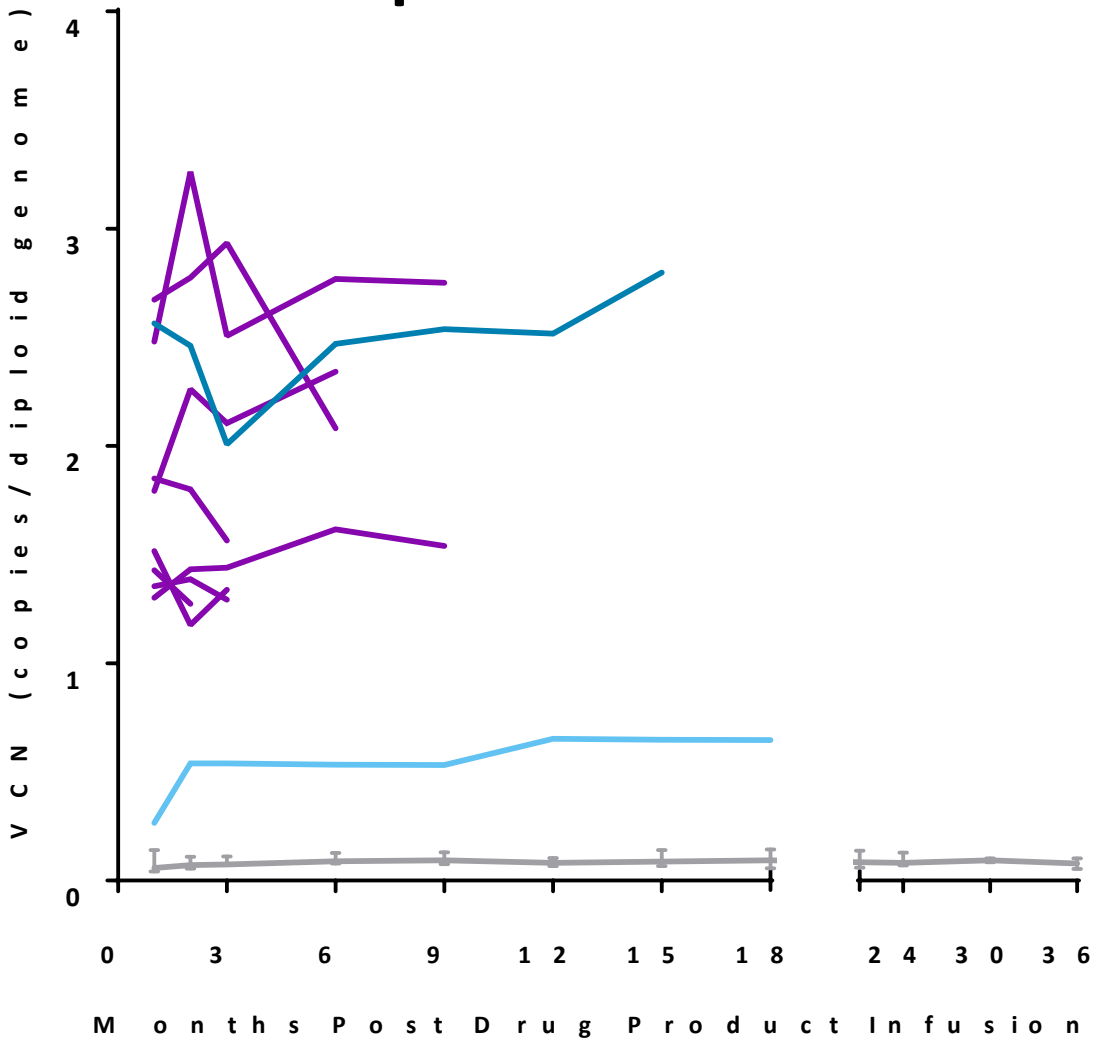
- **Original** (BM)
- **Refined** (BM)
- **Refined** (PB)

†Number of DP exceeds number of patients since some patients were harvested or mobilised more than once; #% Transduced cells not available for 1 DP at time of analyses; ‡1 Group B DP lot was made using original manufacturing process, while the other 3 DP lots were made using refined manufacturing process

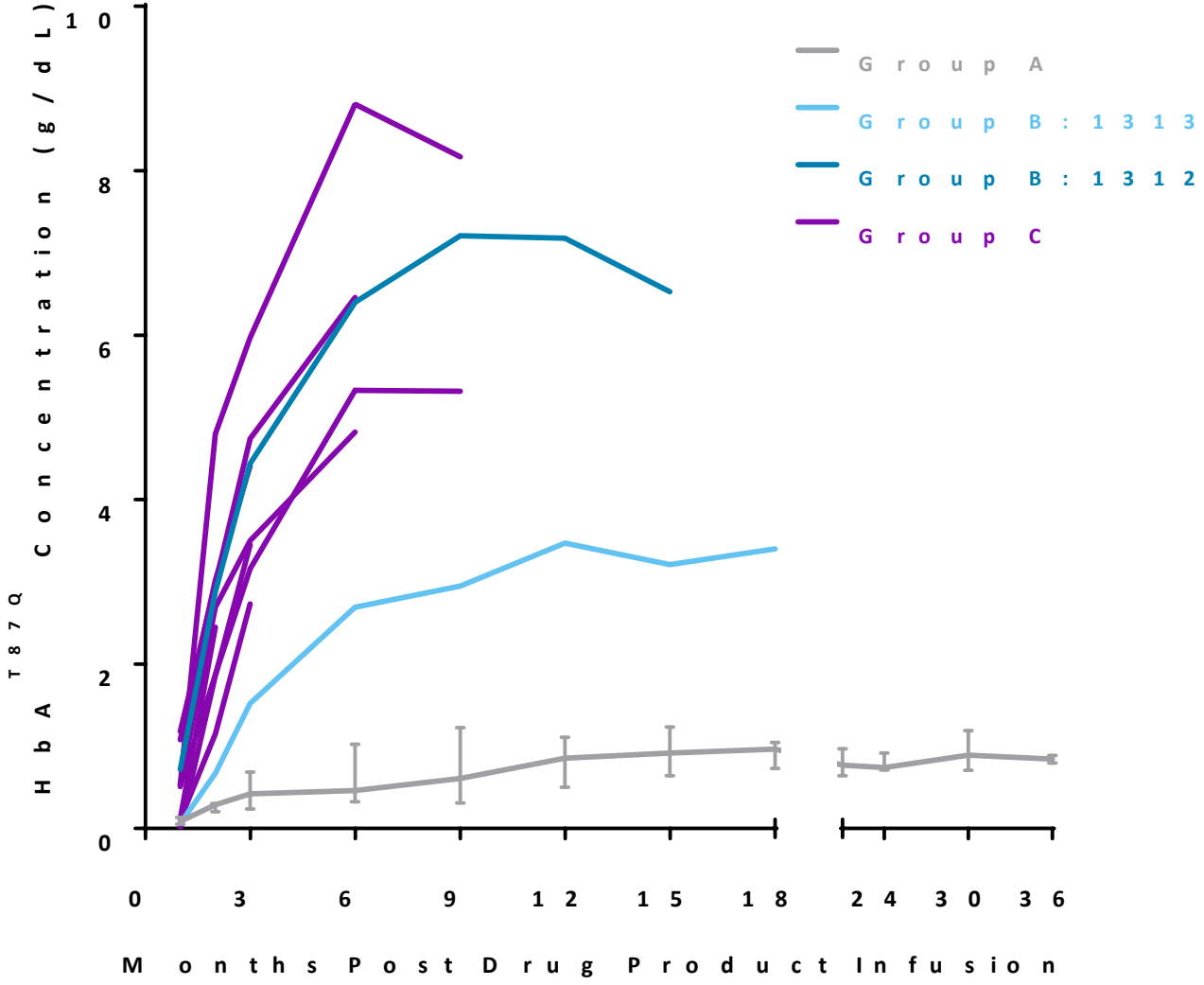
BM, bone marrow; DP, drug product; HSC, haematopoietic stem cell; PB, peripheral blood; VCN, vector copy number

HGB-206: Peripheral blood VCN and HbA^{T87Q} over time

Peripheral blood VCN



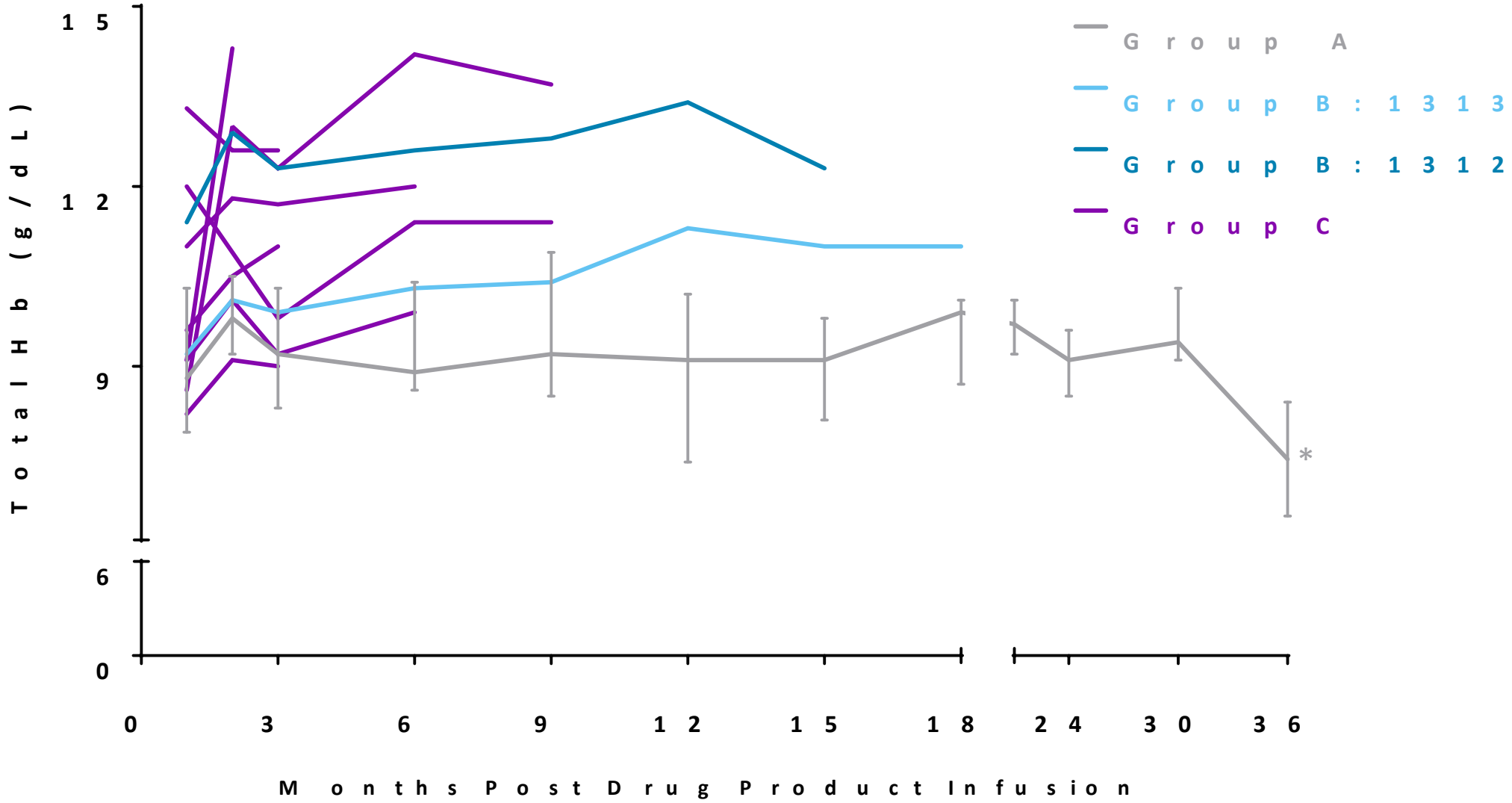
HbA^{T87Q} levels



For Group A patients, medians (Q1, Q3) depicted; Group A patients with month 36 study visit (N=2); Hb, haemoglobin; VCN, vector copy number

Data as of September 14, 2018

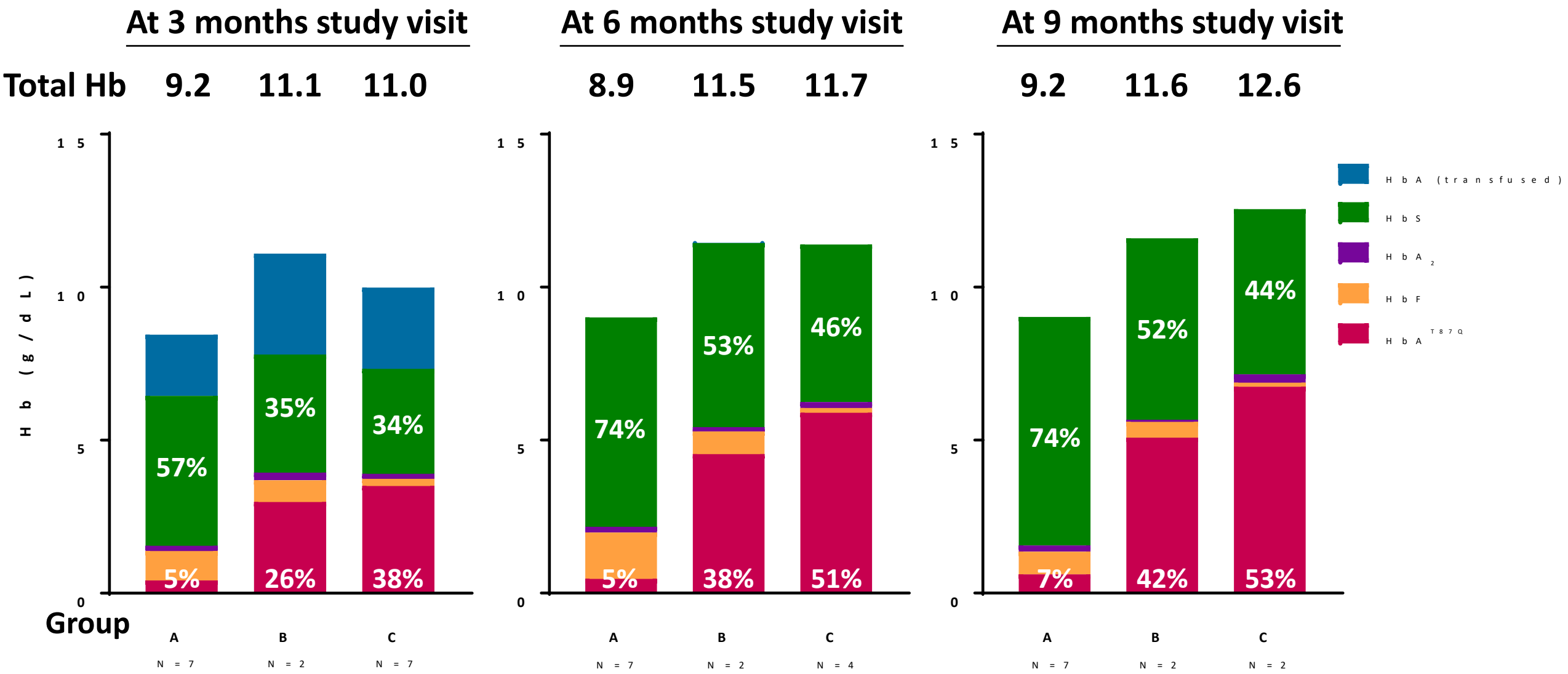
HGB-206: Total Hb levels over time



*N=2 at 36 months

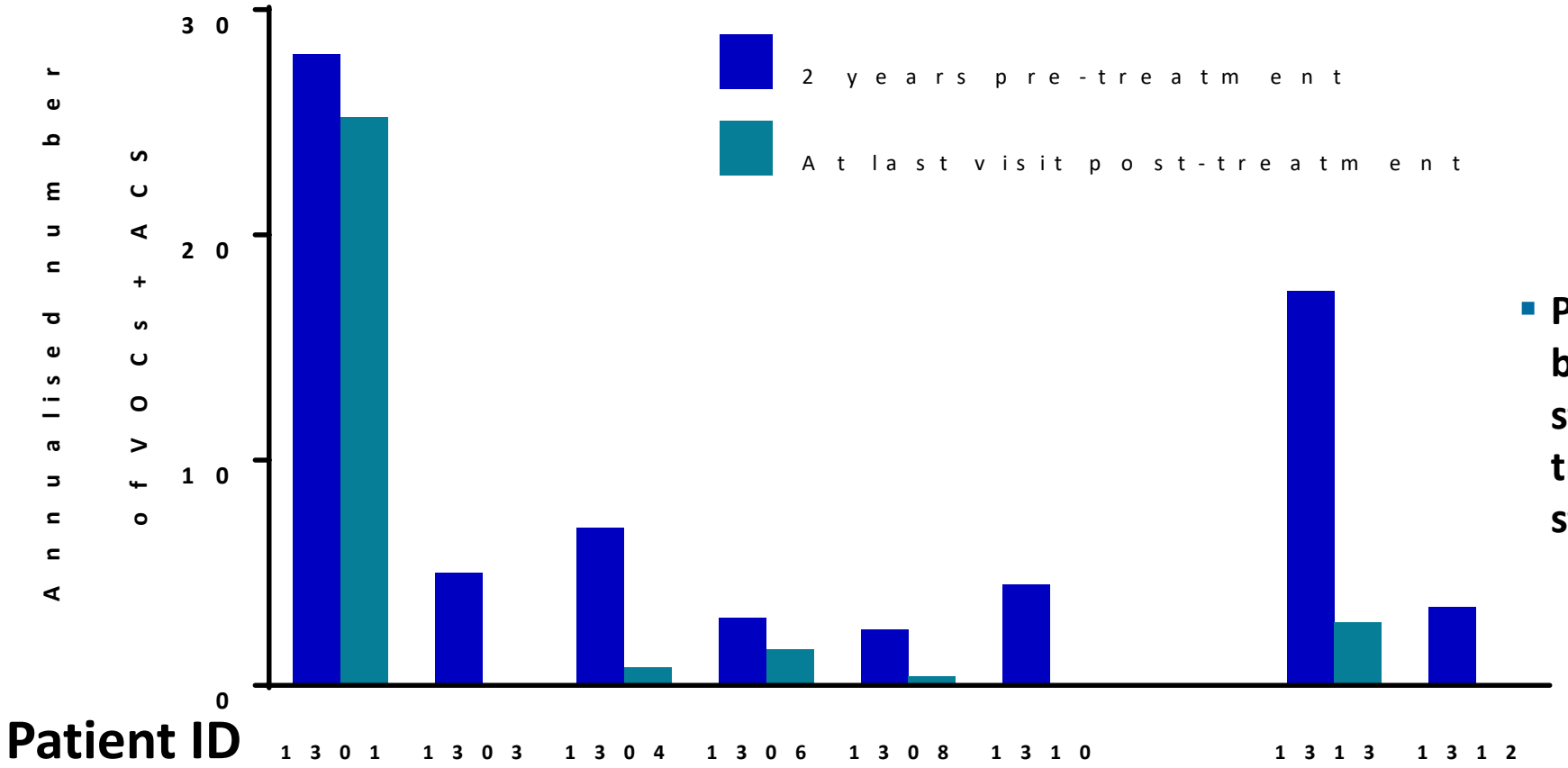
For Group A patients, medians (Q1, Q3) depicted; Hb, haemoglobin

HGB-206: Gene therapy-derived Hb (HbA^{T87Q}) equals or exceeds HbS levels at ≥ 3 months in Group C patients



% represent median Hb fractions as % of total; Hb, haemoglobin

HGB-206 Groups A and B: All patients have decreased rate of annualised VOCs plus ACS post-transplant



- **Patient 1309 with stroke before enrollment stopped RBC transfusions and had no stroke post-infusion**

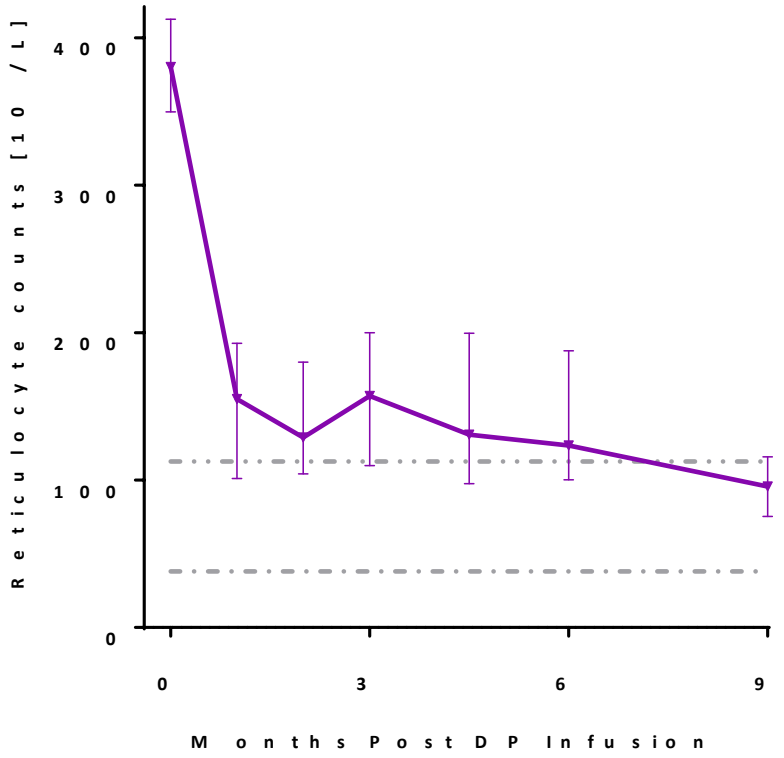
% Decrease from baseline	10	100	89	46	84	100	84	100
Last study visit (Month)	36	36	30	30	30	30	18	15

Investigator-reported adverse events of VOC or ACS are shown

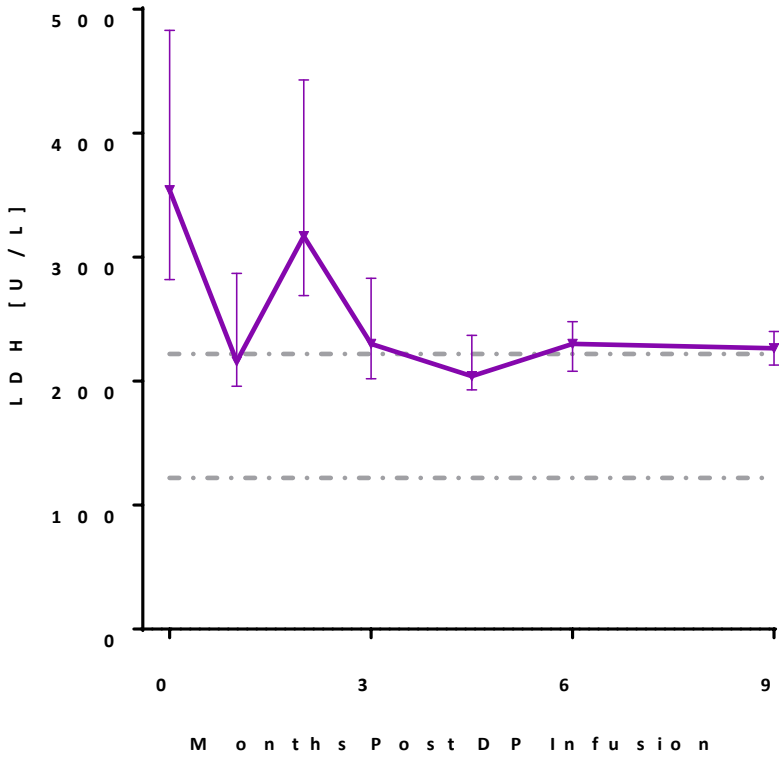
ACS, acute chest syndrome; RBC, red blood cell; VOCs, vaso-occlusive crises

HGB-206 Group C: Decreased haemolysis following LentiGlobin gene therapy

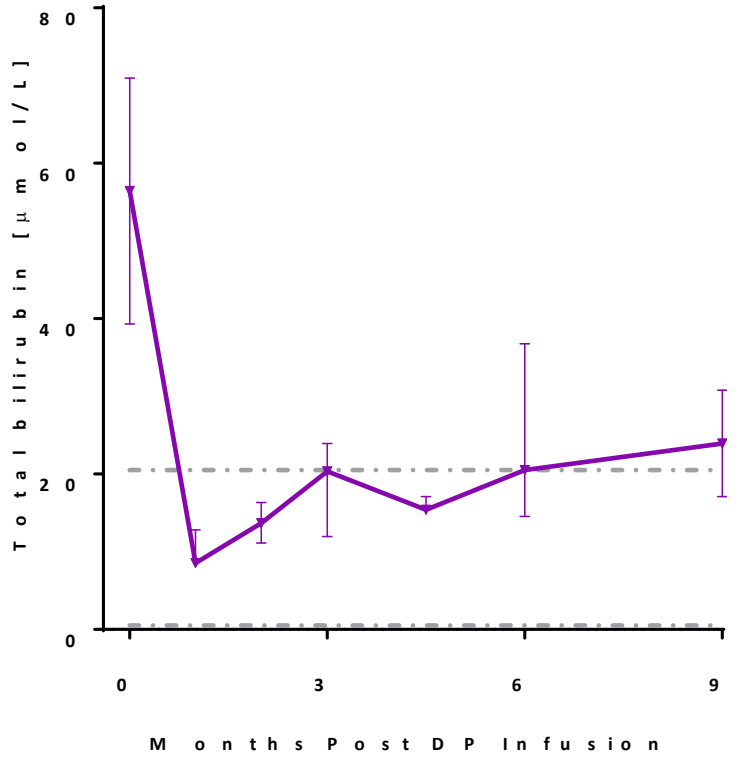
Reticulocyte Counts



Lactate Dehydrogenase



Total Bilirubin



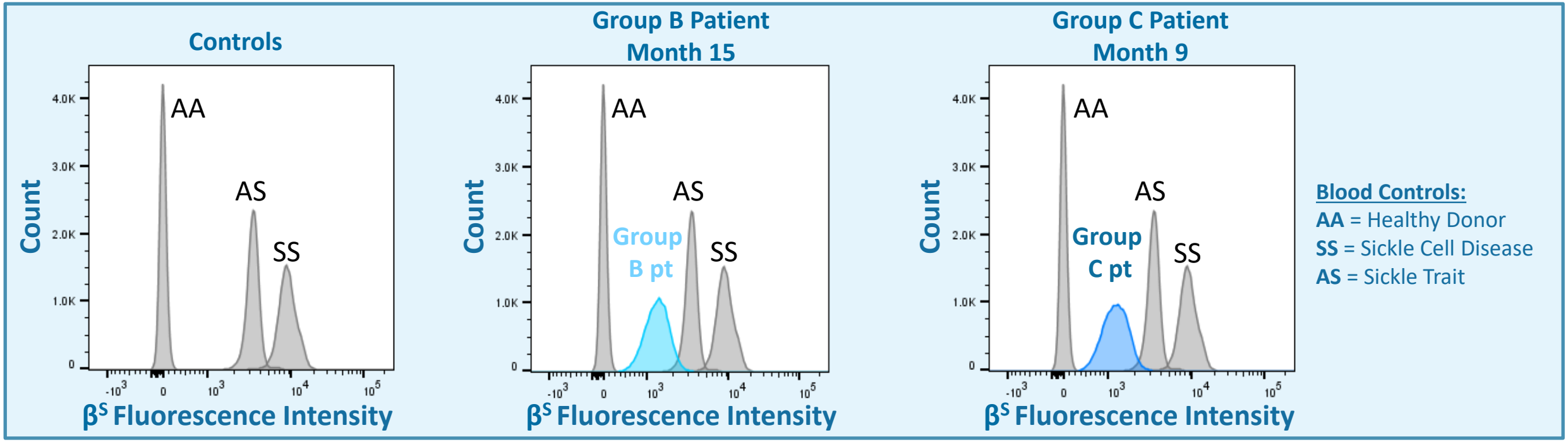
N*							
	4	7	7	7	6	4	2
	7	7	7	7	6	3	2
	9	8	8	7	6	4	2

Median (Q1, Q3) depicted; Dot-dash lines denote lower and upper limits of normal values; *Shows number of patients for whom data are available

DP, drug product; LDH, lactate dehydrogenase

HGB-206: Intracellular staining of RBCs with anti-β^S antibody suggests pancellular distribution of gene therapy-derived HbA^{T87Q} is achievable

- β^S antibody was used for intracellular staining of RBCs followed by FACS analysis
  - Controls showed distinct β^S distributions, with SS > AS > AA
- Results in 2 patients 9 and 15 months post treatment show decreased β^S in nearly all RBCs, even less than AS (trait)
  - Most non-β^S globin in these samples is $\beta^{A\text{-}T87Q}$
  - Patients stopped RBC transfusion and HbF < 2.5% of total globin chains



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

HGB-206: Summary

- Even modest HbA^{T87Q} levels (0.7-2.8 g/dL at last visit) can have clinical effect (reduced VOC plus ACS frequency)
- Refined manufacturing and other protocol modifications have improved results:
  - **Both Group B patients had increased HbA^{T87Q} levels (3.4 and 6.5 g/dL) and total Hb levels associated with 84 and 100% reduction in frequency of VOCs and ACS**
  - **Group C demonstrates robust HbA^{T87Q} production of 4.8-8.8 g/dL at ≥ 6 months that equals or exceeds HbS levels**
    - Safety and feasibility of plerixafor mobilisation and aphaeresis in SCD was shown
    - Hb of 9.9-13.7 g/dL at last visit without RBC transfusion
    - Decreased haemolysis after LentiGlobin gene therapy
- Safety profile of LentiGlobin gene therapy for severe SCD is consistent with myeloablative conditioning and underlying SCD
  - One case of MDS reported, not related to LentiGlobin gene therapy
- Exploratory translational assay suggests pancellular expression of gene therapy-derived Hb
- Protocol amended to further evaluate the clinical impact of LentiGlobin gene therapy in SCD

ACS, acute chest syndrome; Hb, haemoglobin; MDS, myelodysplastic syndrome; RBC, red blood cell; VOC, vaso-occlusive crisis

Updates to HGB-206: An open-label, multicentre phase 1/2 study of LentiGlobin for severe sickle cell disease

Enrollment Criteria: Group C

- ≥ 12 and ≤ 50 years of age
- At least 4 severe VOEs* in the 24 months prior to consent
- Failure or intolerance to hydroxyurea
- Any history of severe cerebral vasculopathy† leads to exclusion

Target enrollment: 35 evaluable subjects

Study Endpoints: Group C

- Primary:
  - Globin Response
    - Weighted average HbA^{T87Q} ≥ 30% of total Hb AND
    - Weighted average total Hb increase of ≥ 3 g/dL compared to baseline total Hb OR weighted average total Hb ≥ 10 g/dL
- Key Secondary:
  - A 75% reduction in severe VOEs in 24 months following DP infusion

*VOEs include acute episodes of pain, acute chest syndrome, hepatic sequestration, splenic sequestration, or priapism (priapism considered as long as medical attention was needed); †Defined as overt or haemorrhagic stroke, abnormal transcranial Doppler [≥200 cm/sec] needing chronic transfusion, or occlusion or stenosis in the polygon of Willis, or presence of Moyamoya disease

DP, drug product; Hb, haemoglobin; VOE, vaso-occlusive event

Thank you to the study participants and their families

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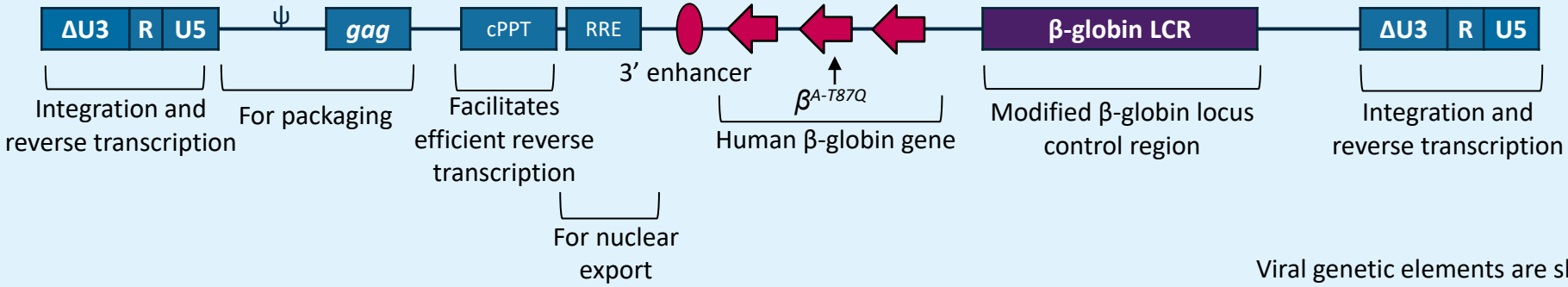
- Iva Kronja

Back-up

BB305 vector and LentiGlobin Drug Product

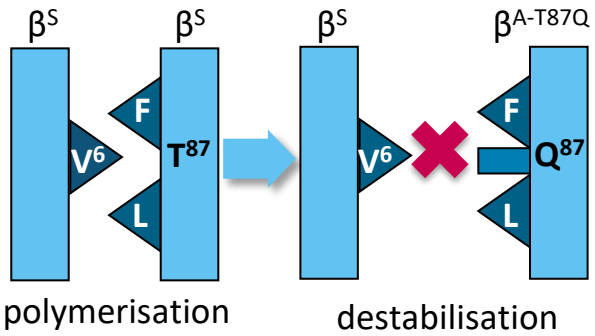
BB305 lentiviral vector



Viral genetic elements are shown in blue

BB305 Vector

- BB305 (betibeglogene darolentivec) is a self-inactivating lentiviral vector which is replication incompetent[1,2]
- The vector was designed to express a modified human β-globin gene (*HBB*) with an anti-sickling mutation, T87Q[1,2]
  - This mutation was designed to incorporate the anti-sickling properties of HbF via a threonine to glutamine substitution at position 87[1,3]
- The modified *HBB* gene encodes for production of the transgenic protein, β^{T87Q} which is incorporated into HbA^{T87Q} [1]
  - HbA^{T87Q} inhibits HbS polymerisation
  - β^{T87Q} can be distinguished from other globin chain variants by reverse-phase HPLC



Kanter. ASH 2015. Abstract 3233

LentiGlobin Drug Product

- Autologous CD34+ cells are transduced with the BB305 lentiviral vector to create LentiGlobin Drug Product

HbA, adult haemoglobin; HbS, sickle haemoglobin; HbF, fetal haemoglobin; HPLC, high performance liquid chromatography.

1. Negre et al. Hum Gene Ther. 2016; 2. Negre et al. Curr Gene Ther. 2015; 3. Pawliuk et al. Science 2001.