#206

The Relationships Between Target Gene Transduction, Engraftment of HSCs and RBC Physiology in Sickle Cell Disease Gene Therapy

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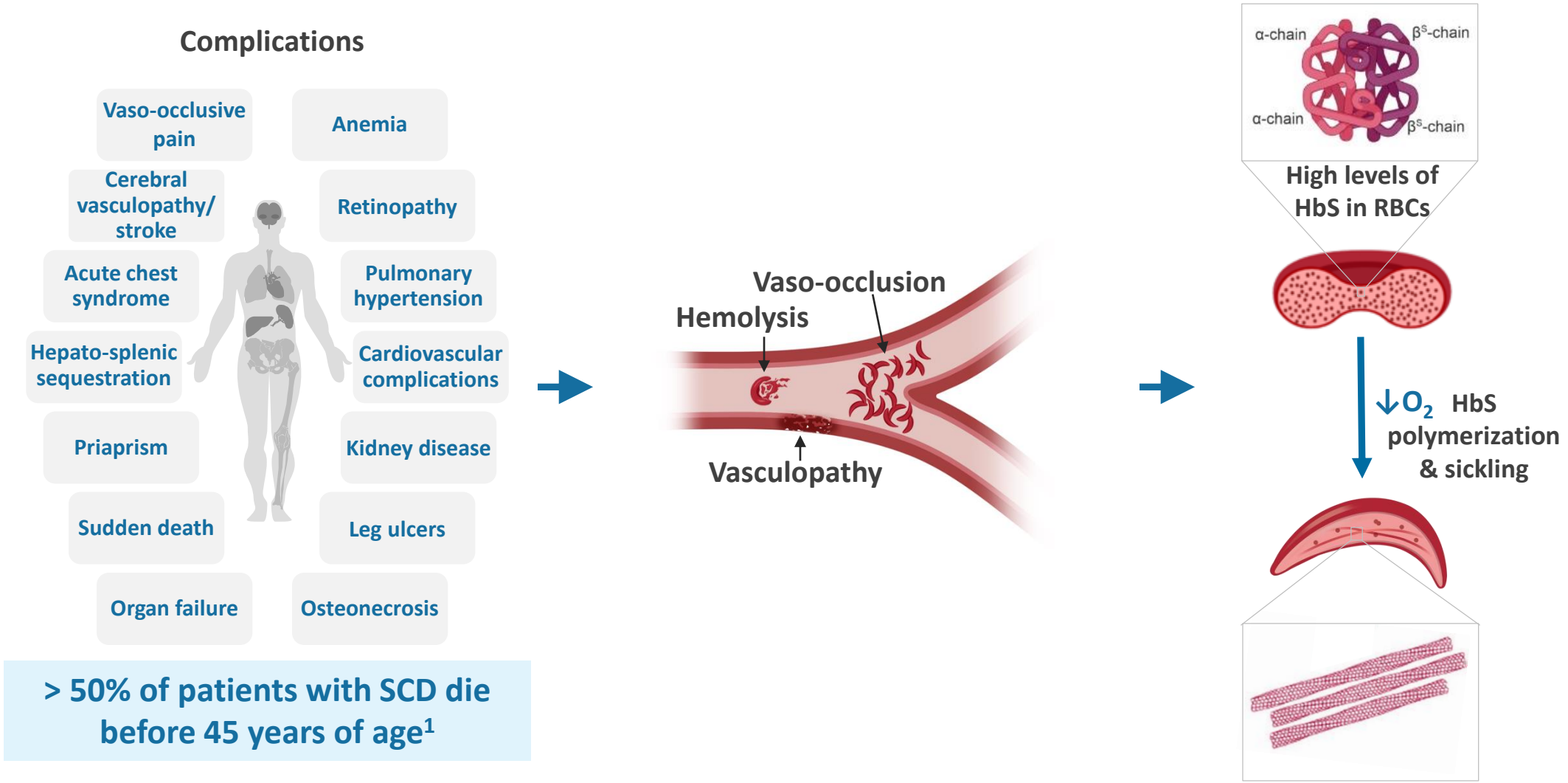
Sickle cell disease (SCD) is characterized by high morbidity and early mortality

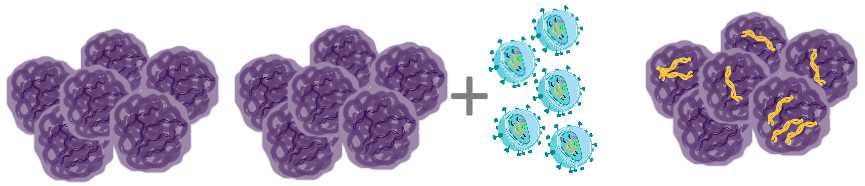


1. Hassell K., Am J Prev Med, 2010

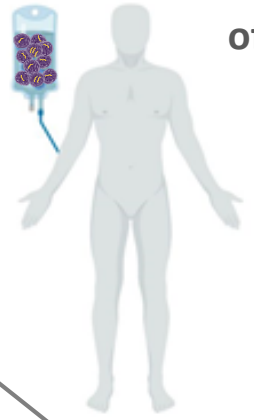
Hb, hemoglobin; RBC, red blood cell

LentiGlobin for SCD gene therapy overview

LentiGlobin DP centralized manufacturing

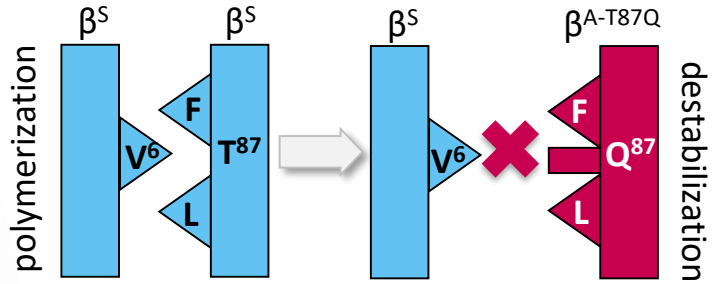


Select CD34+ HSCs | *Transduce with BB305 lentiviral vector* | *Cryopreserve, test, release DP*



Transduced HSCs engraft & contribute to reconstitution of functional RBCs

Modified RBCs express gene therapy-derived, anti-sickling HbA^{T87Q}

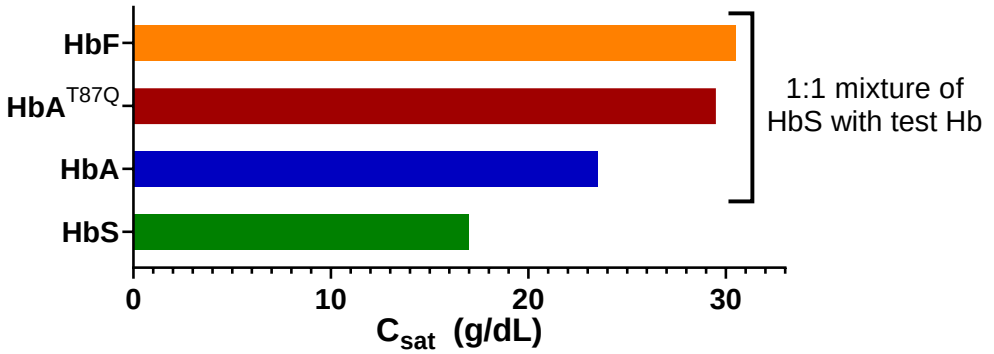


	Group A	Group B	Group C
Pre-collection transfusion regimen	Optional	Required	Required
HSC source	Bone marrow	Bone marrow	Mobilized PB
Manufacturing process	Original	Orig → Refined	Refined

Abstract #2061 **Abstract #990**

Sat, Dec 7, 5:30-7:30 pm at the Orange County Convention Center, Hall B

HbA^{T87Q} inhibits HbS polymerization *in vitro* similarly to HbF and has comparable O_2-binding affinity to HbA

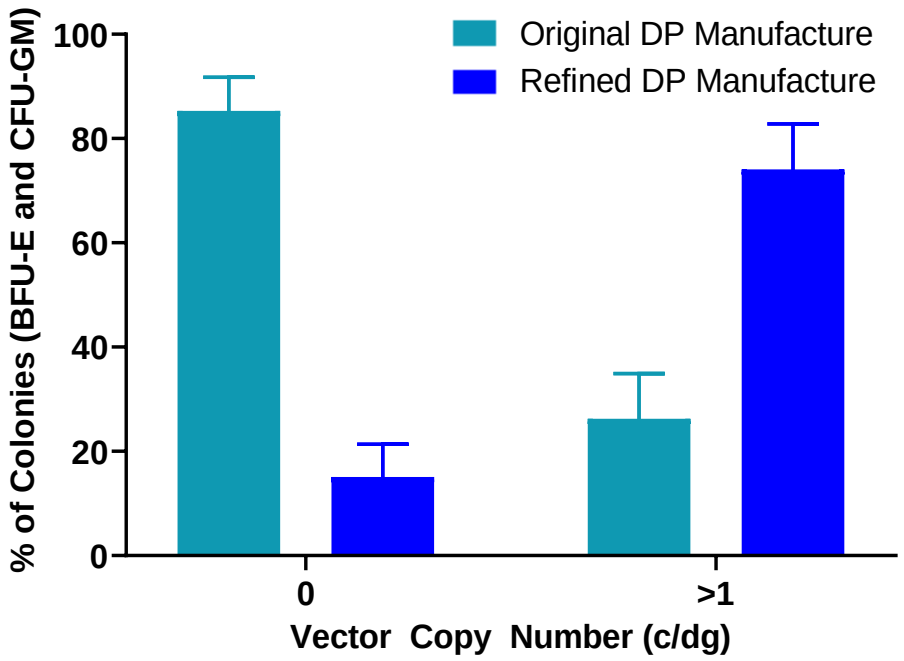


Pawliuk et al., Science 2001

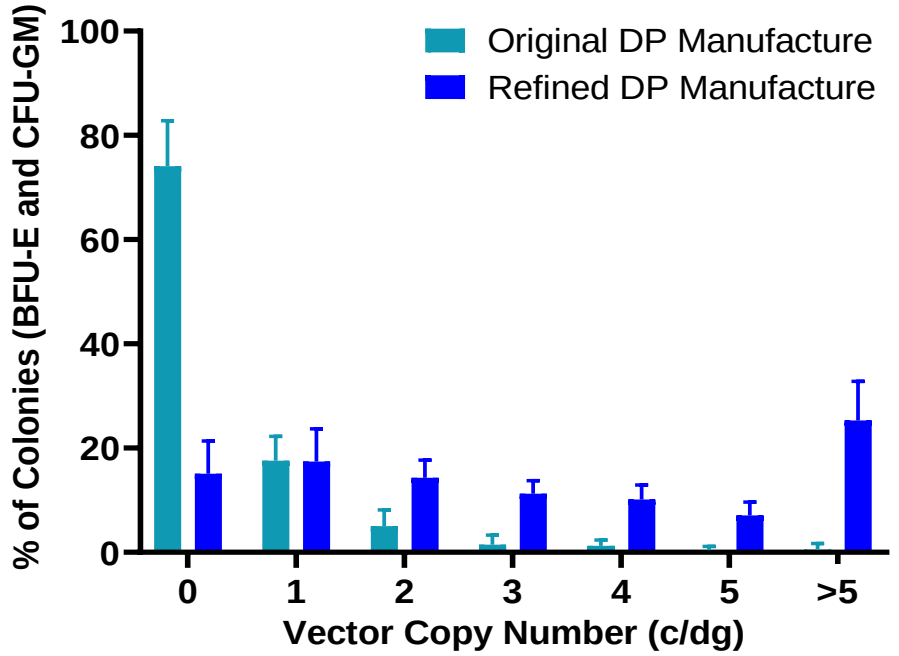
DP, drug product; HSCs, hematopoietic stem cells; PB, peripheral blood; RBC, red blood cell

Impact of protocol and manufacturing changes on VCN in drug product and peripheral blood

Higher %LVV+ colonies with the refined vs original manufacturing process



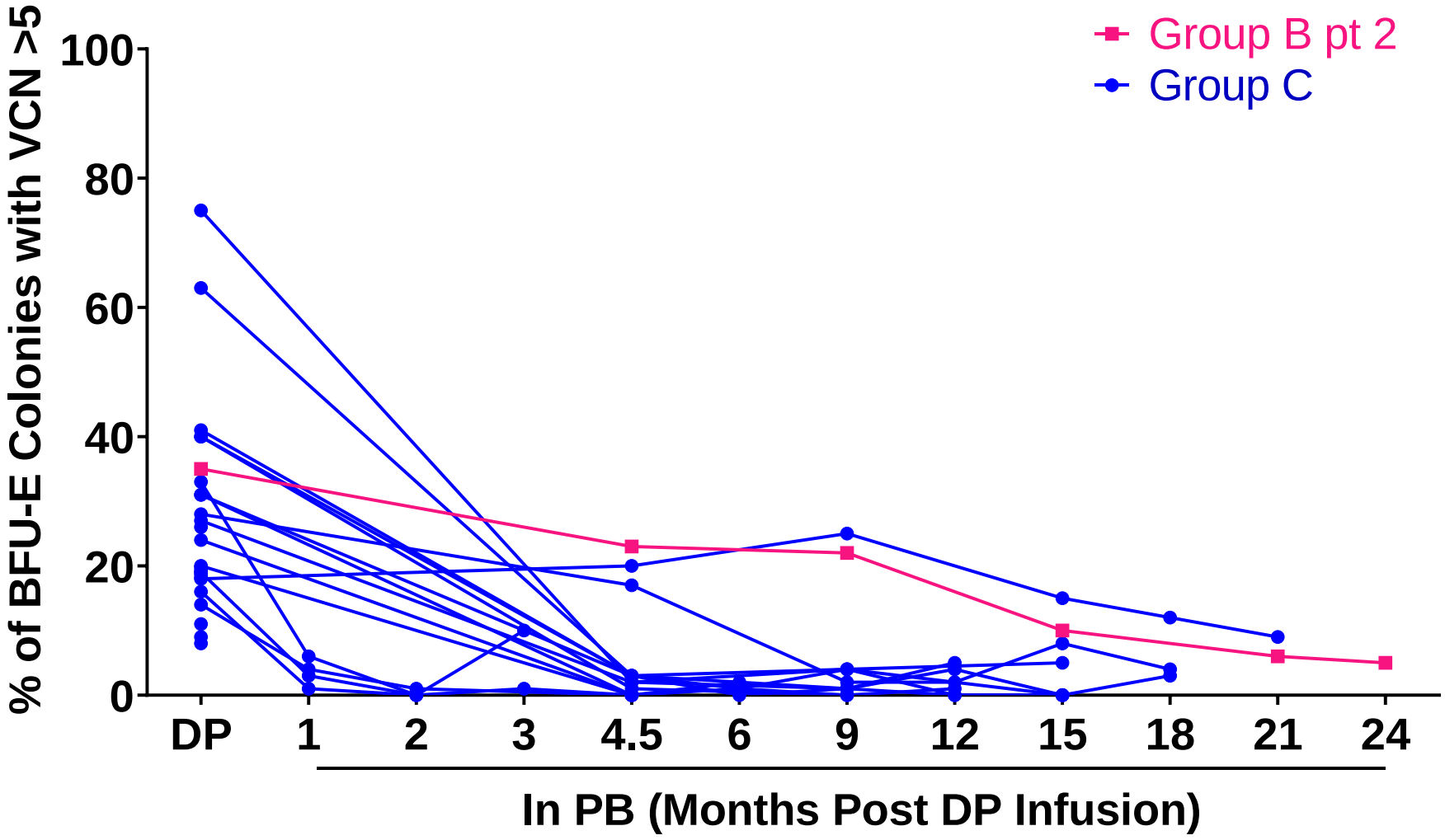
Refined manufacture results in increased %LVV+ and greater contribution of high-copy cells



Do these high-copy cells engraft long-term?

BFU-E, burst forming unit-erythroid; c/dg, copy/diploid genome; CFU-GM, colony forming unit granulocyte, monocyte; DP, drug product; LVV, lentiviral vector; VCN, vector copy number

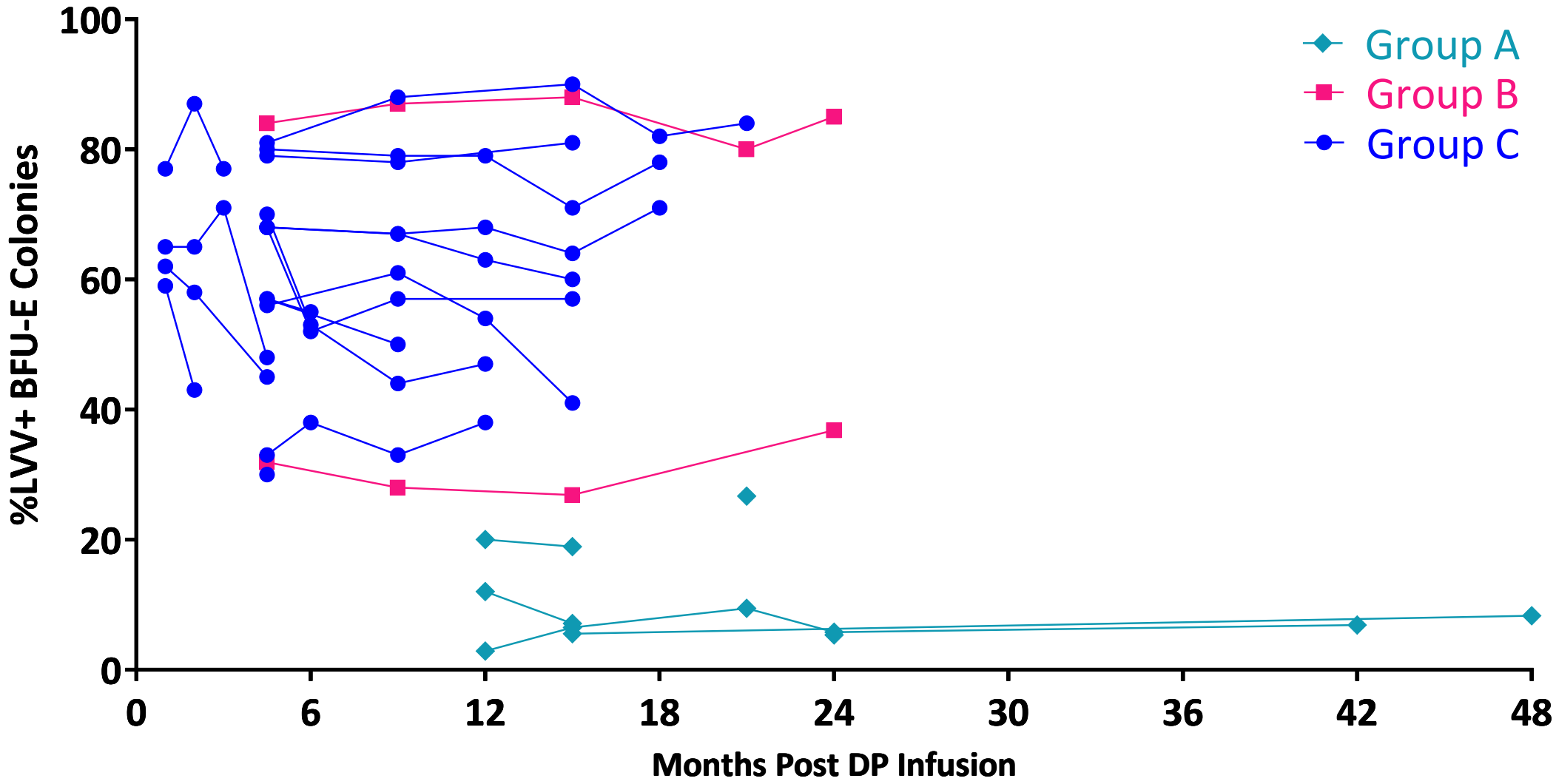
Lack of persistence of high-VCN containing cells



Each line represents one patient (n=1 Group B and n=16 Group C)

BFU-E, burst forming unit-erythroid; DP, drug product; PB, peripheral blood; VCN, vector copy number

Refined protocol results in higher %LVV+ in peripheral blood and LVV+ cells demonstrate stable engraftment

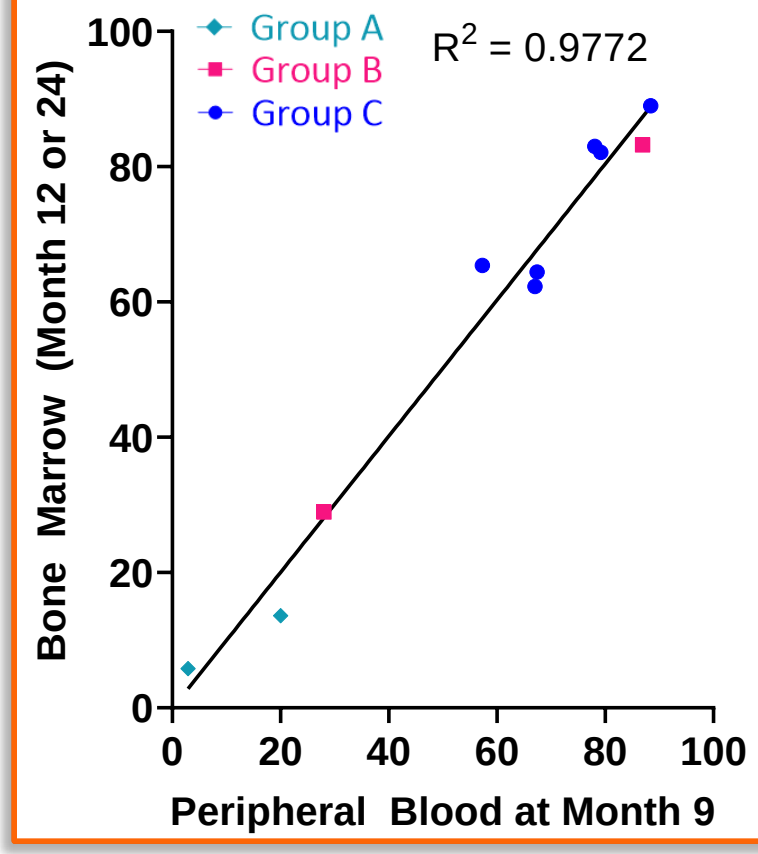


Each patient (n=6 Group A, n=2 Group B and n=17 Group C) is represented by a unique symbol or a unique combination of colored line and symbol

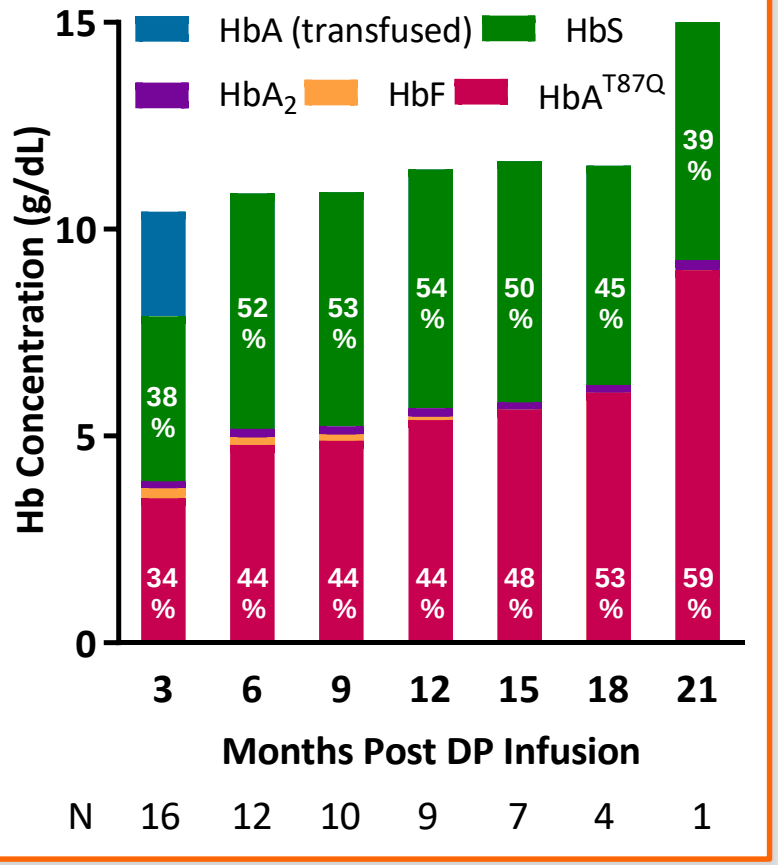
BFU-E, burst forming unit-erythroid; DP, drug product; LVV, lentiviral vector

%LVV+ in the peripheral blood correlates with both bone marrow engraftment and HbA^{T87Q} expression

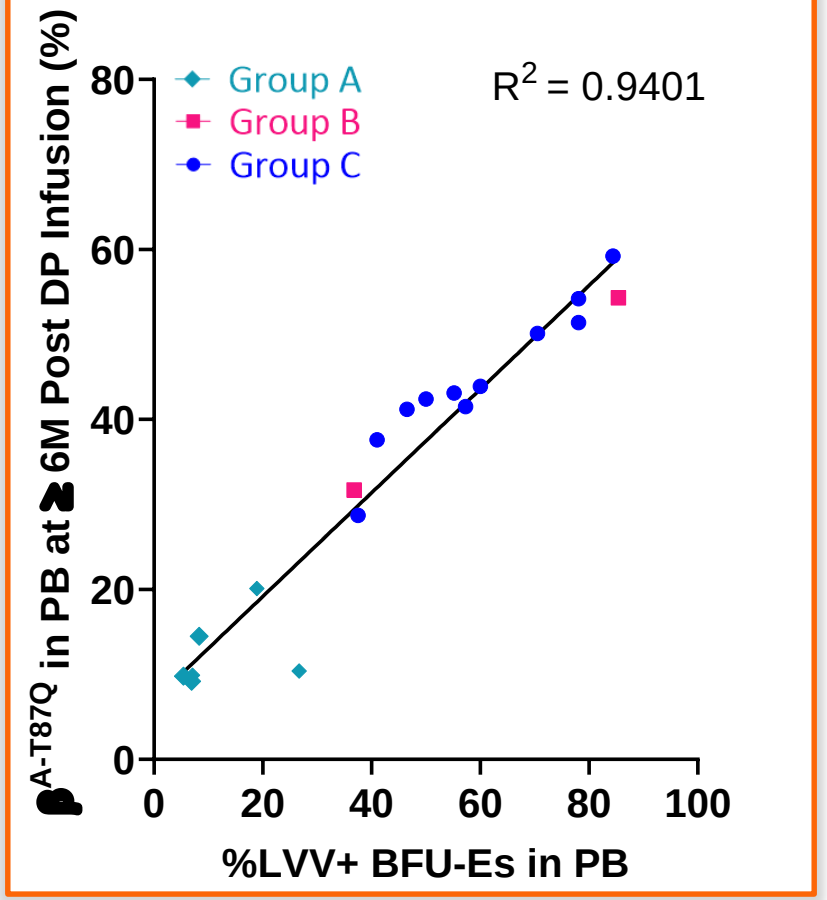
%LVV+ in PB is a good proxy for BM LVV-marking



Median Hb fractions in PB of Group C subjects over time



%LVV+ BFU-E colonies from PB correlates well with $\beta^{A\text{-}T87Q}$ expression

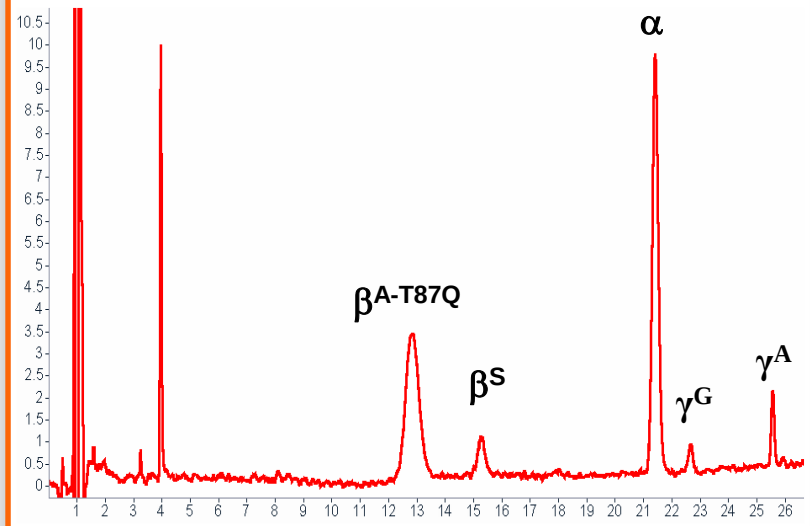


BFU-E, burst forming unit-erythroid; BM, bone marrow; DP, drug product; Hb, hemoglobin; LVV, lentiviral vector; PB, peripheral blood

Effect of VCN on $\beta^{A\text{-}T87Q}$ and β^{S} levels in PB-derived erythroid colonies

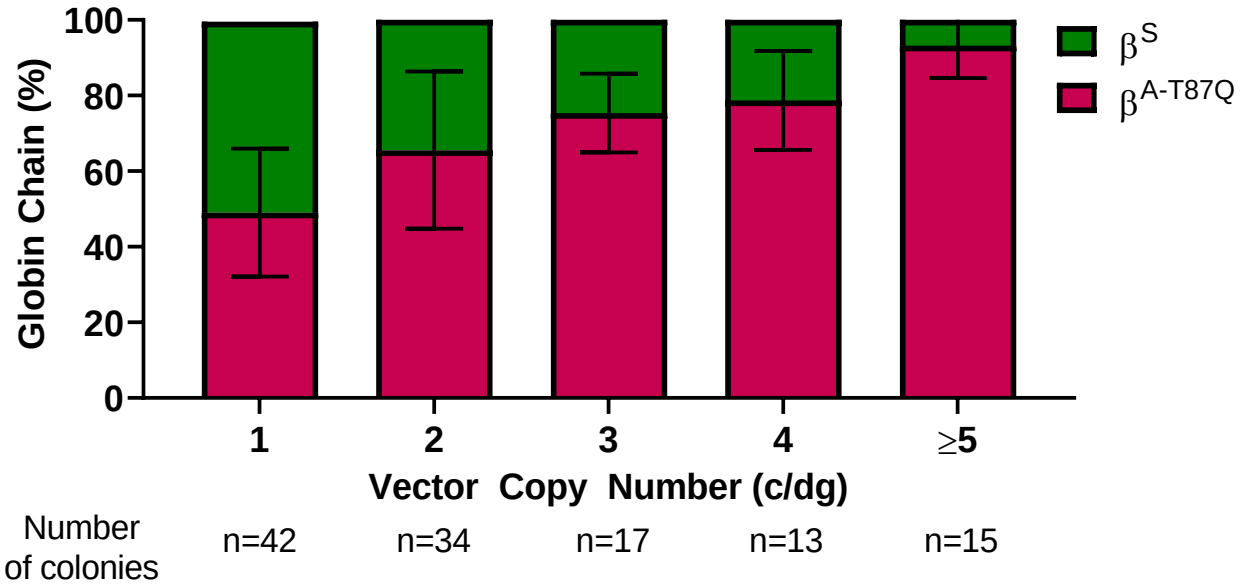
Sample ratio of all β-globin chains

Example for VCN = 3



Production of $\beta^{A\text{-}T87Q}$ results in a reduction in β^{S}-globin in BFU-E colonies irrespective of the level of HbA^{T87Q} production

Average globin chain % for the 3 representative subjects with different levels of HbA^{T87Q}



Number of colonies	n=42	n=34	n=17	n=13	n=15

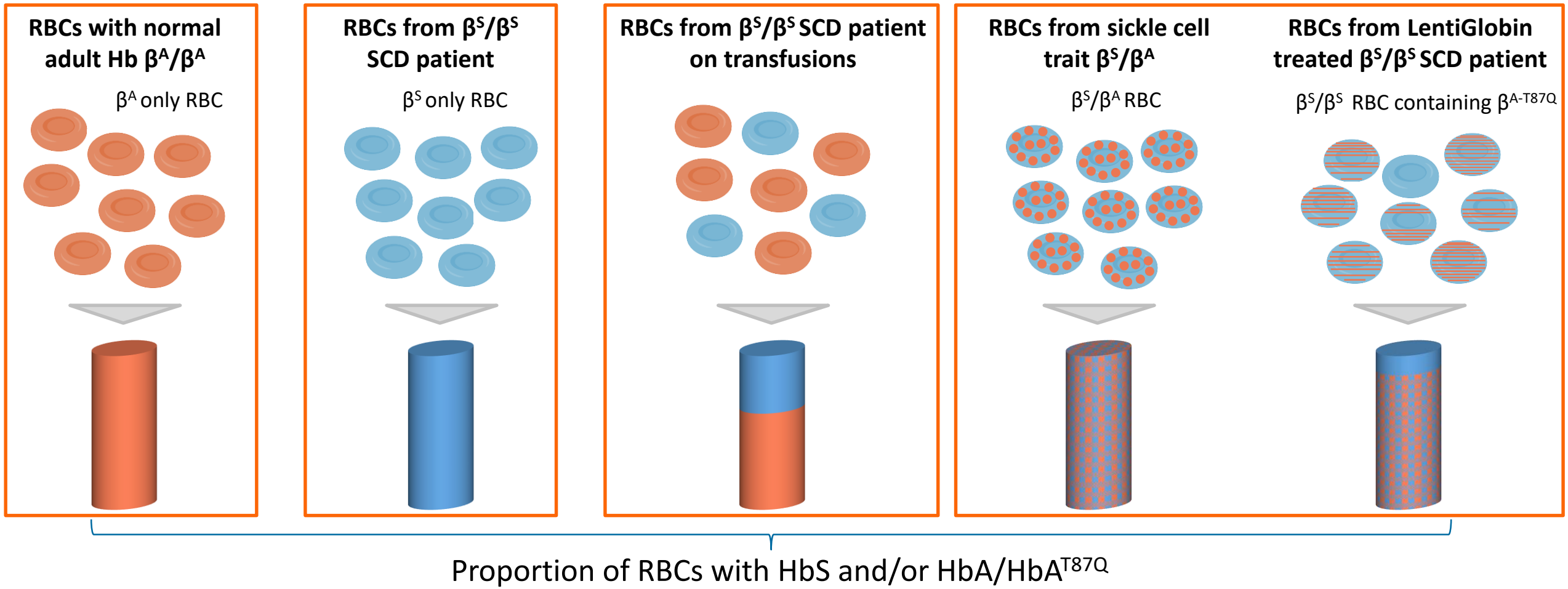
- HbA^{T87Q} levels in these three subjects at the time of analysis post DP infusion were 3.6 – 8.8 g/dL

- Single BFU-E colonies cultured from PB samples isolated post DP infusion were processed to:
  - Measure VCN
  - Separate globin chains by UPLC

BFU-E, burst forming unit-erythroid; c/dg, copies per diploid genome; DP, drug product; PB, peripheral blood; UPLC, ultra performance liquid chromatography; VCN, vector copy number

Pancellularity of HbA^{T87Q}: Exploratory assay allows for single-cell resolution of Hb expression

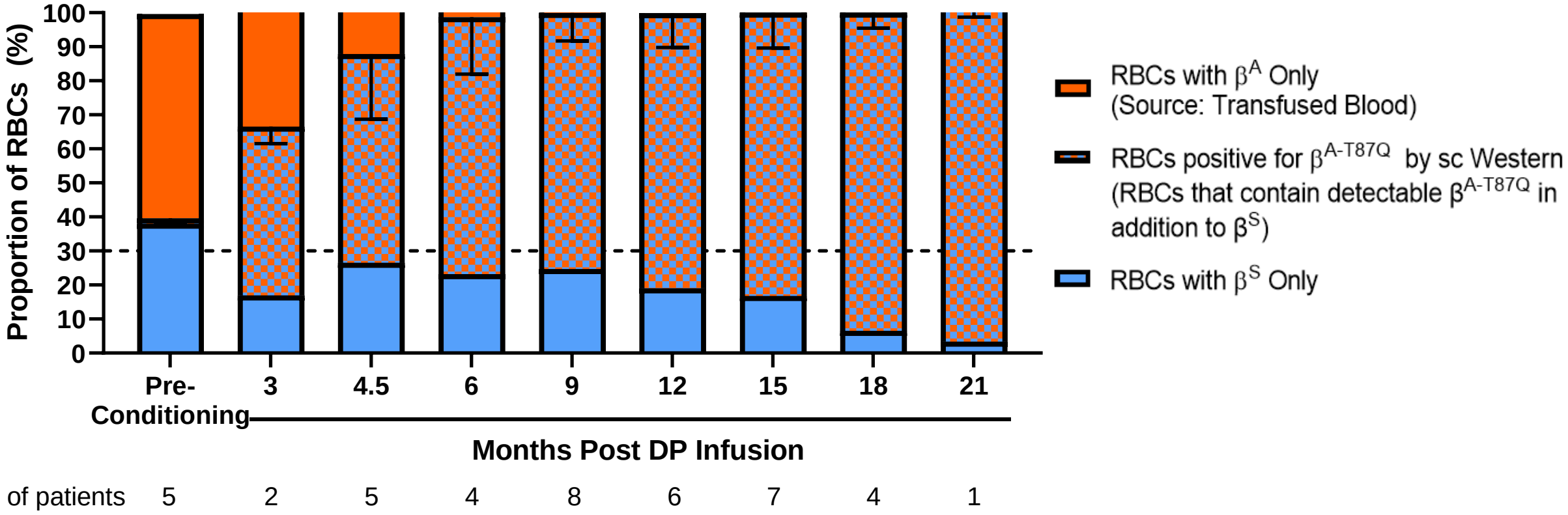
- Single red blood cell western with anti-β^S or anti-$\beta^A/\beta^{A\text{-}T87Q}$ antibodies



Hb, hemoglobin; RBC, red blood cells

On average, ≥ 70% of RBCs from patients treated with LentiGlobin contain $\beta^{A\text{-}T87Q}$ by Month 6

- Single RBC western assay was performed in multiple patient samples

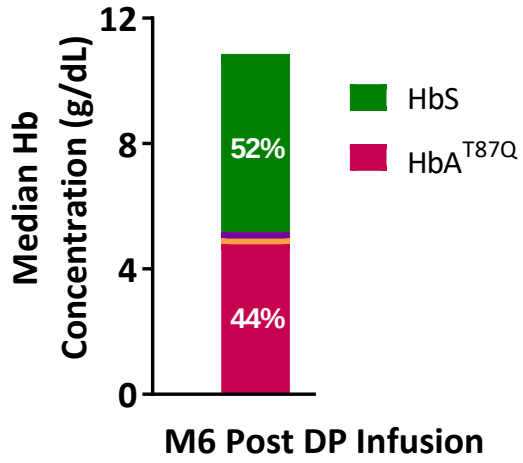


Mean ± SD is depicted - if N=1, data show technical replicates; *Pre-conditioning sample does not contain any $\beta^{A\text{-}T87Q}$, signal is due to error rate of multiples

DP, drug product; RBCs, red blood cells; sc, single cell; SD, standard deviation

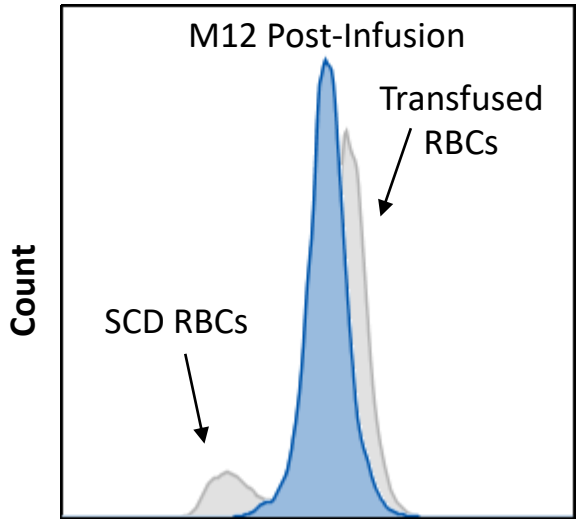
HbA^{T87Q} levels in RBCs from patients post DP infusion are estimated to be comparable to the amount of HbA per RBC in sickle cell trait

Stable MCH in Group C despite higher $\beta^{A\text{-}T87Q}$ further suggests decrease in β^{S}

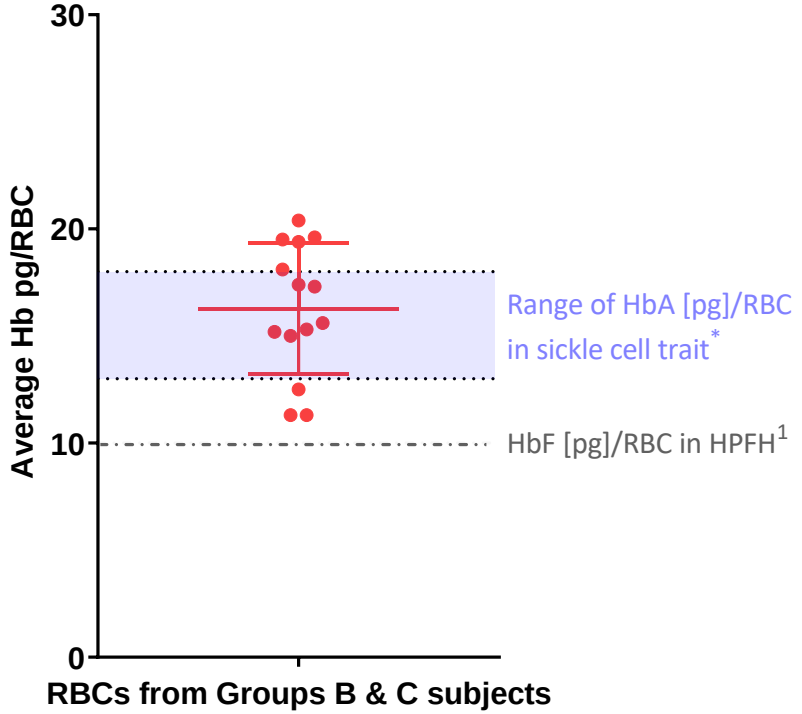


Median MCH	
Baseline (n=30)	M6 post DP infusion (n=12)
31.6 pg	31.6 pg

Uniform distribution of $\beta^{A\text{-}T87Q}$



HbA^{T87Q} pg/RBC comparable to HbA pg/RBC in trait & HbF pg/RBC in HPFH



Average Hb pg/RBC = (% HbA^{T87Q} of total Hb/% RBCs containing $\beta^{A\text{-}T87Q}$) x MCH

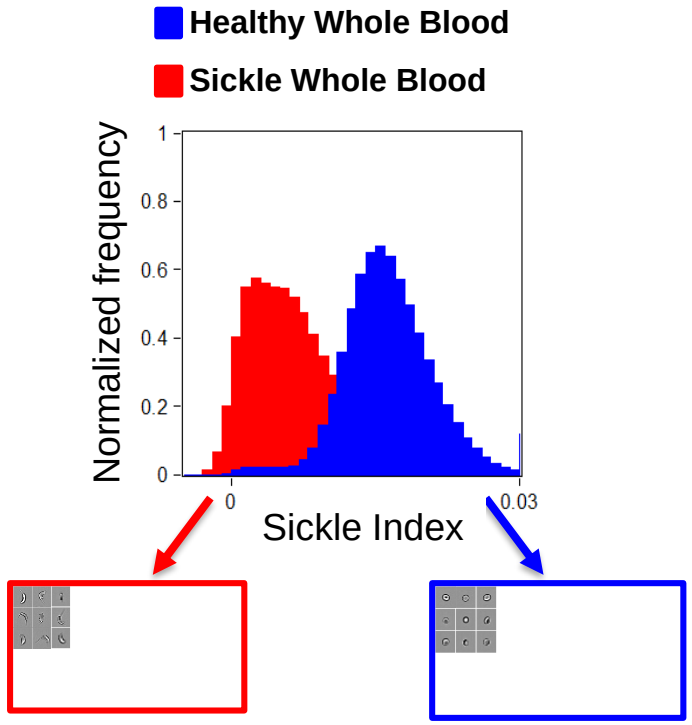
*Calculated using 50% HbA/RBC for the lower end of the range and 60% HbA/RBC for the upper end of the range

1. Steinberg MH et al., Blood. 2014;123(4):481-5.

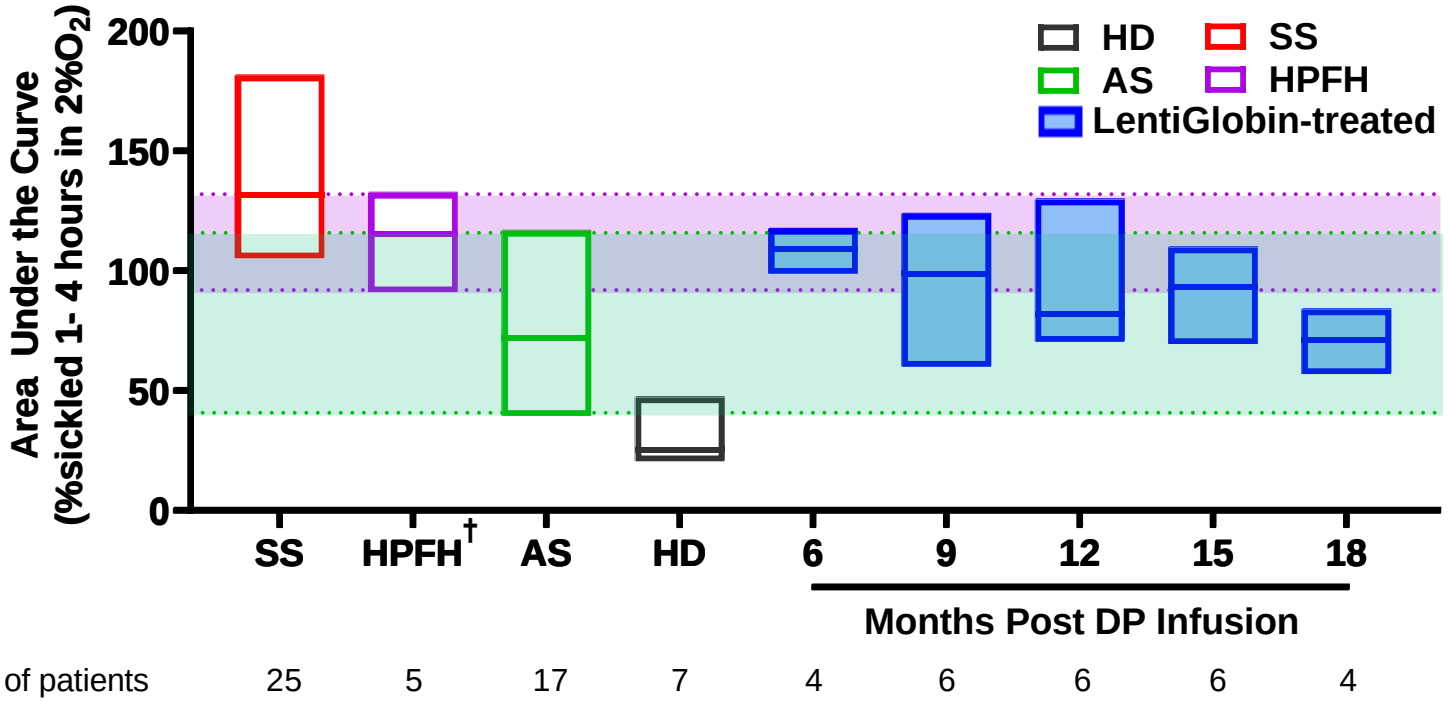
DP, drug product; Hb, hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; MCH, mean corpuscular hemoglobin; RBC, red blood cells

Impact of intracellular $\beta^{A\text{-}T87Q}$ and β^{S} levels on RBC sickling

Assay to distinguish sickled RBCs



% sickling for LentiGlobin-treated patients with ≥ 6M follow-up*



Propensity to sickle decreases over time post-gene therapy with LentiGlobin; Group C similar to trait

*Group C only; †HbF contribution to total Hb in these samples ranged from 28% – 42%

AS, sickle cell trait; DP, drug product; HD, healthy donor; HPFH, hereditary persistence of fetal hemoglobin; SS, sickle mutation on both *HBB* alleles

Summary

- Exploratory assays provide important new information demonstrating that revisions to manufacturing process and treatment protocols have improved the transduction, engraftment, and transgene expression profile of LentiGlobin HSC gene addition therapy for SCD
  - Largely stable presence of %LVV+ BFU-E colonies from PB ≥ ~4.5 Months post DP infusion, but lack of long-term engraftment of colonies with VCN >5
  - Assessment of gene marking can be done with PB as proxy for BM marking
  - Near pancellular (≥ 70% of RBCs) expression of HbA^{T87Q} after ≥ 6M post DP infusion
  - Expression of $\beta^{A\text{-}T87Q}$ results in a reduction in β^{S} levels as demonstrated by MCH stability
  - With an average of ~16 pg of HbA^{T87Q} per cell, RBCs from treated patients post DP infusion are comparable to the amount of HbA per RBC in sickle cell trait
  - Expression of HbA^{T87Q} results in a reduction in sickling propensity down to levels seen in sickle cell trait

BFU-E, burst forming unit erythroid; DP, drug product; HSC, hematopoietic stem cell; MCH, mean corpuscular hemoglobin; PB, peripheral blood; RBC, red blood cells; VCN, vector copy number

Thank you to the study participants and their families

Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago
Children's Hospital of Philadelphia, Philadelphia
Columbia University Medical Center, NYC
Emory University, Atlanta
Medical University of South Carolina, Charleston
NIH, Molecular and Clinical Hematology Branch, Bethesda
UCSF Benioff Children's Hospital, Oakland
University of Alabama at Birmingham, Birmingham

Later today: *Sat, Dec 7, 5:30-7:30 pm*
at the Orange County Convention Center, Hall B

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

Abstract #2061 Abstract #990

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