ASGCT 2020 Abstract #972

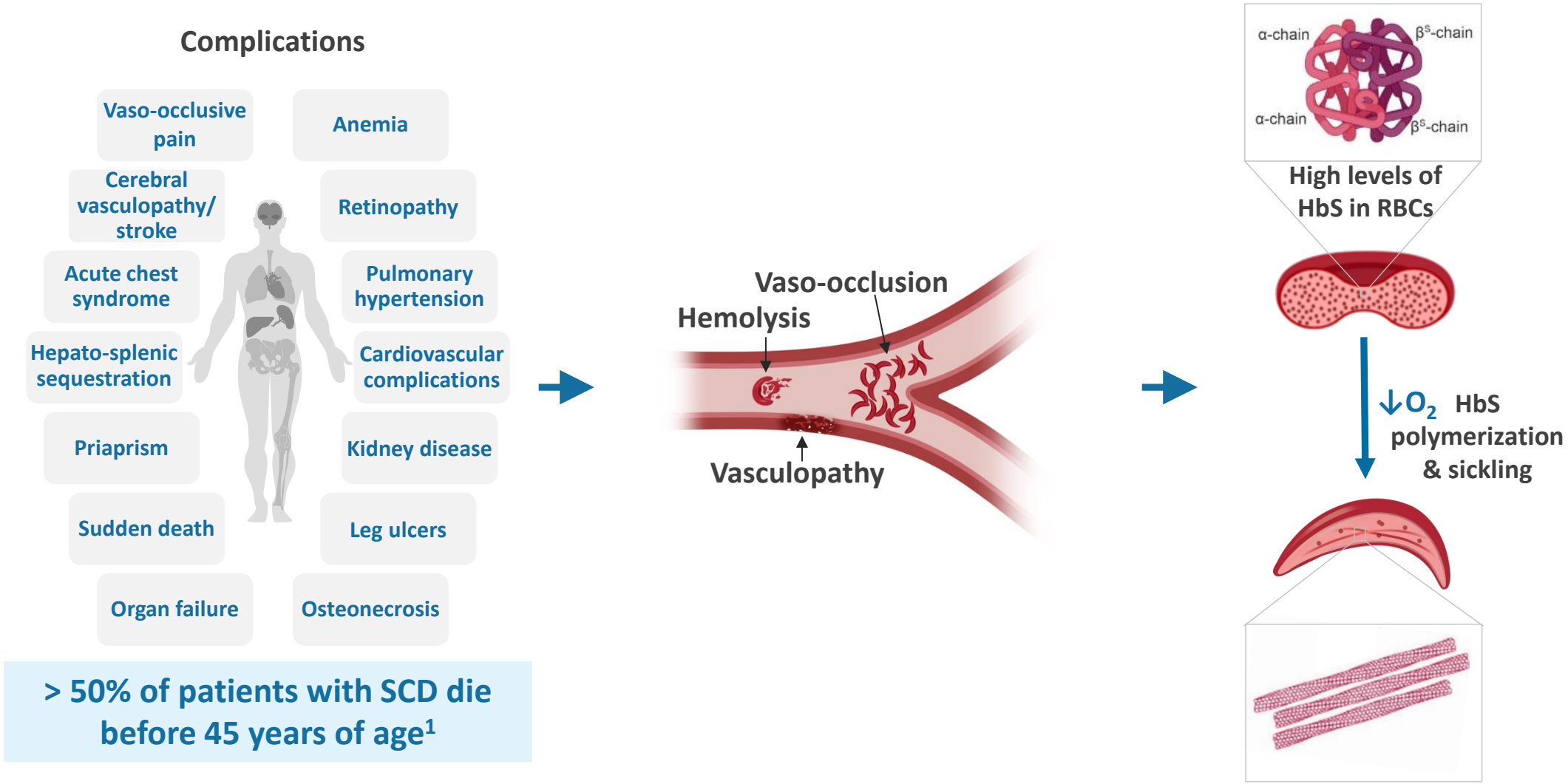
# Gene Transduction, Hematopoietic Stem Cells (HSCs) Engraftment, and Red Blood Cell (RBC) Physiology in Sickle Cell Disease (SCD) Gene Therapy

Melissa Bonner, Julie Kanter, Elizabeth Macari, Ricky Lane, Gretchen Lewis, Paige Coles, Sarah Kassenaar, Sai Mynampati, Robert Schulze, Madison Hebert, Mark C. Walters, Alexis A. Thompson, Mohammed Asmal, John F. Tisdale and Francis J. Pierciey Jr.

# ASGCT 2020

- Disclosure of Affiliations: Francis J. Pierciey Jr
- Employment and Stock Ownership: bluebird bio, Inc.

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

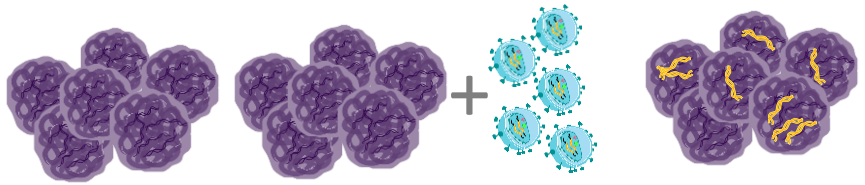



Hb, hemoglobin; RBC, red blood cell

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

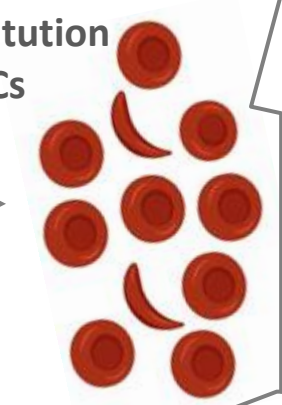
# LentiGlobin for SCD gene therapy overview

## LentiGlobin DP centralized manufacturing

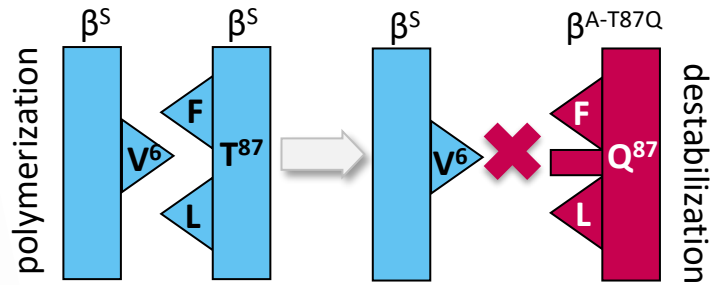


*Select CD34+ HSCs* | *Transduce with BB305 lentiviral vector* | *Cryopreserve, test, release DP*

## Transduced HSCs engraft & contribute to reconstitution of functional RBCs



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

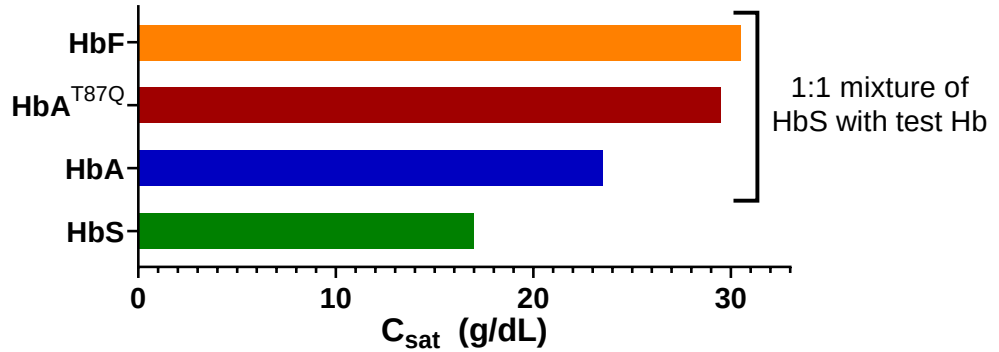



| | Group A | Group B | Group C |
|---|---|---|---|
| Pre-collection transfusion regimen | Optional | Required | Required |
| HSC source | Bone marrow | Bone marrow | Mobilized PB |
| Manufacturing process | Original | Orig → Refined | Refined |

**Abstract #1298**

Fri, May 15, 8:15-8:30 am in the Clinical Trials Spotlight Symposium session

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

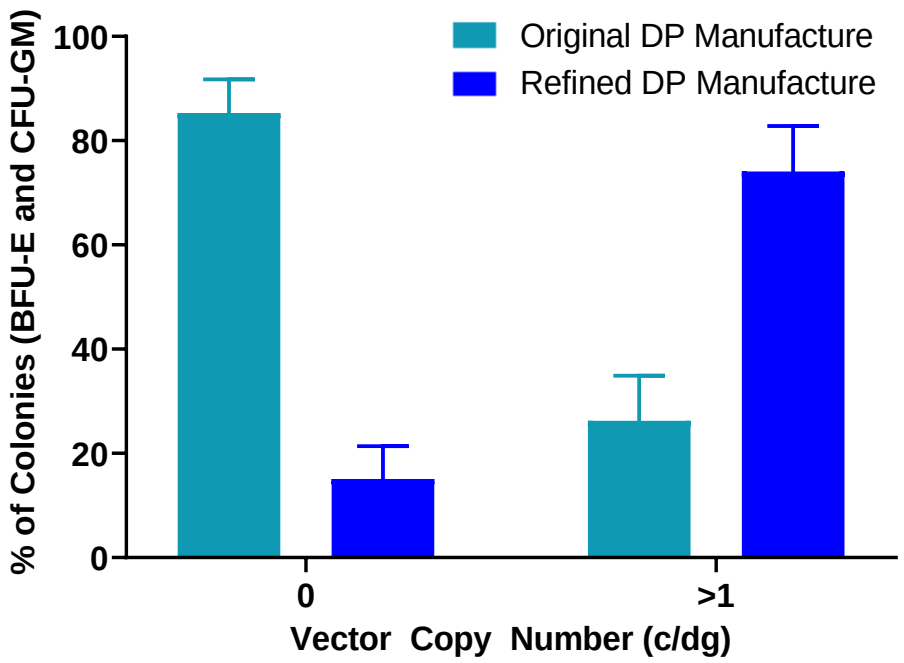



Pawliuk et al., Science 2001

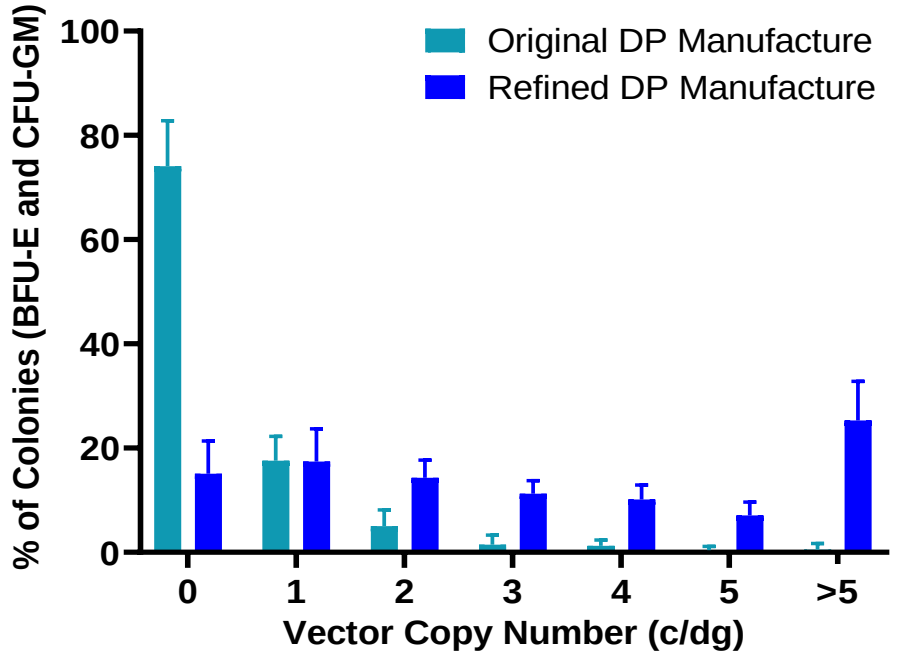
DP, drug product; HSCs, hematopoietic stem cells; PB, peripheral blood; RBC, red blood cell

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

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



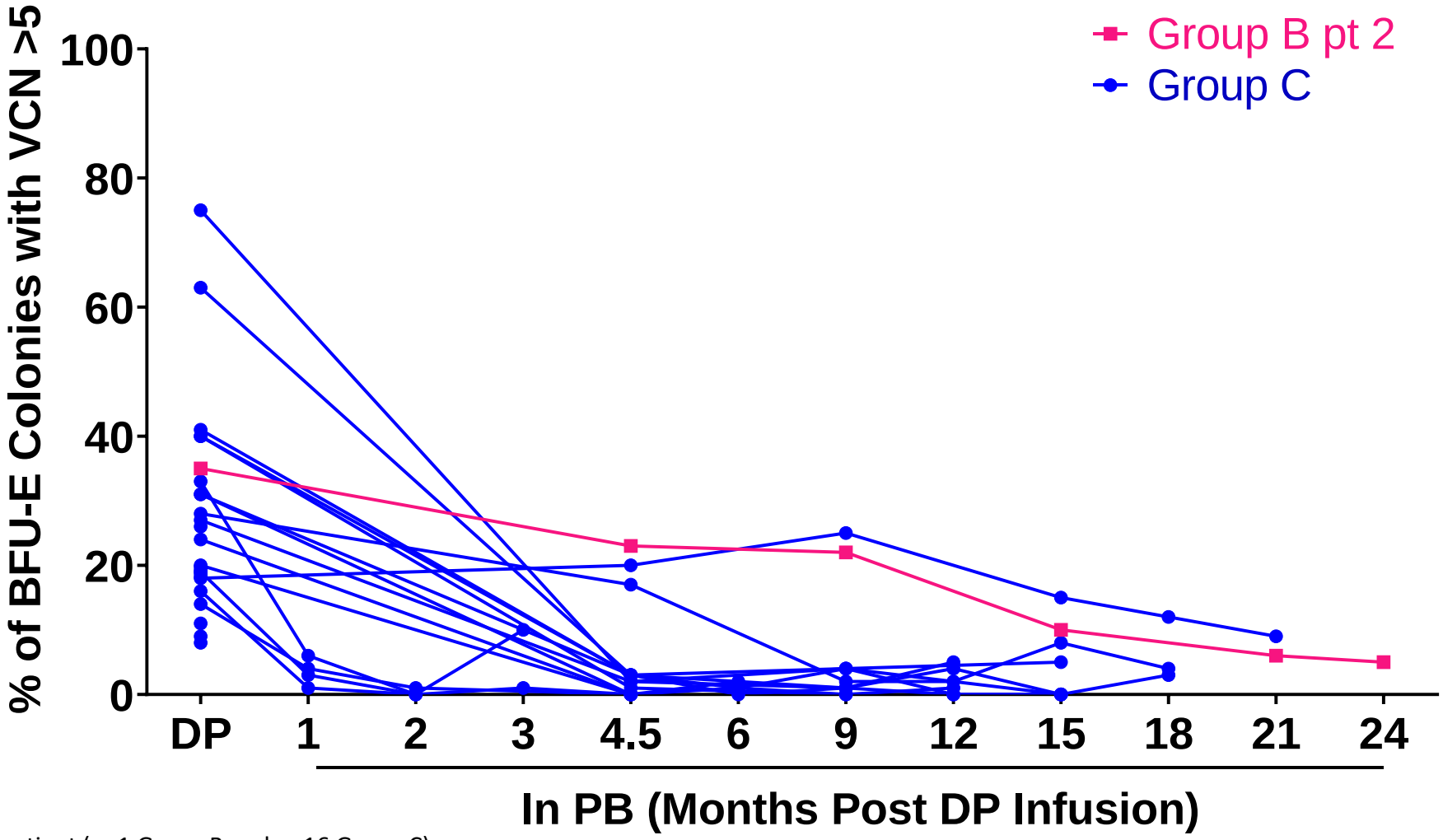

## Refined manufacture results in increased %LVV+ and greater contribution of high-copy cells




**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

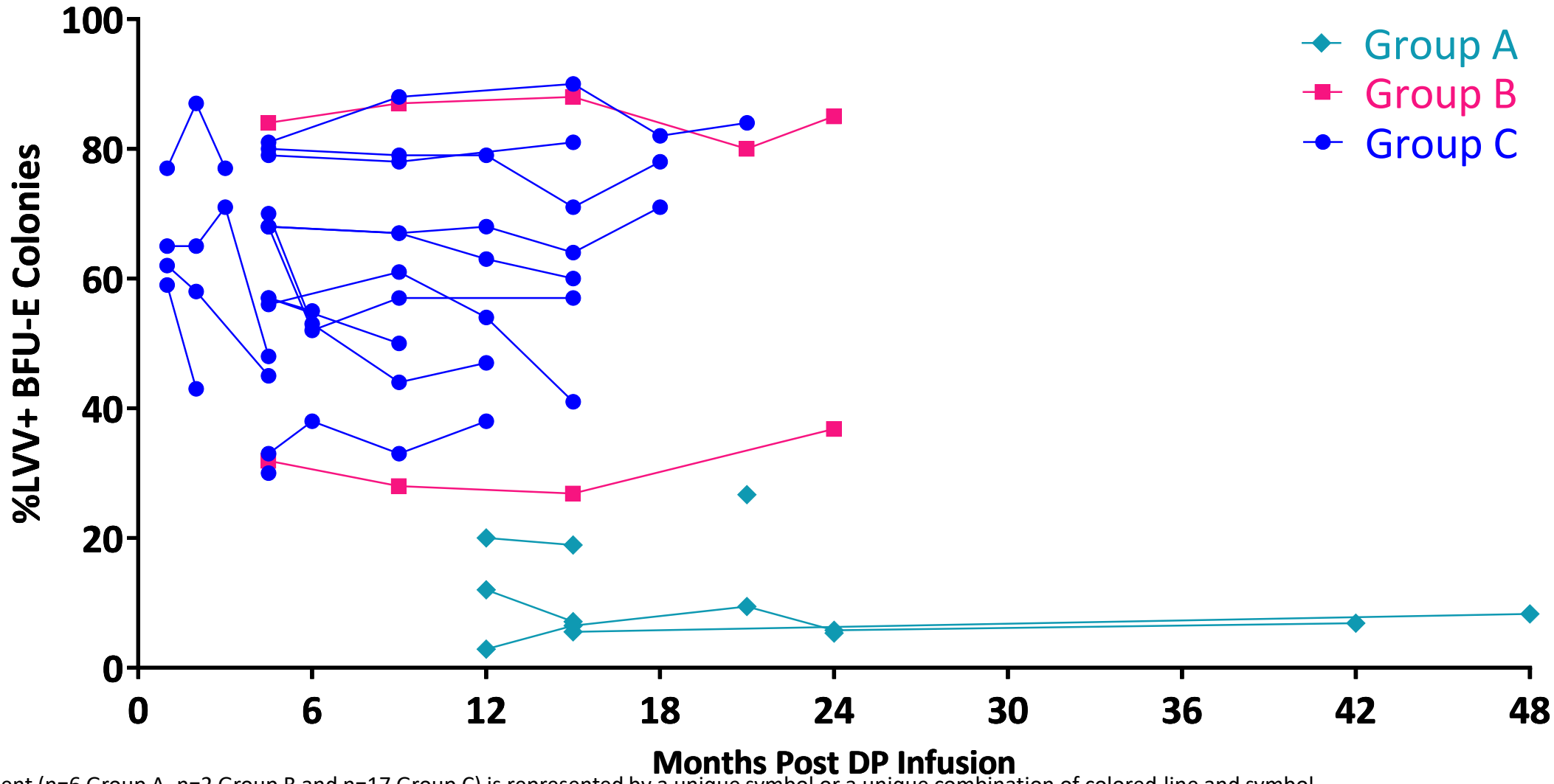
# Lack of persistence of high VCN-containing cells




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

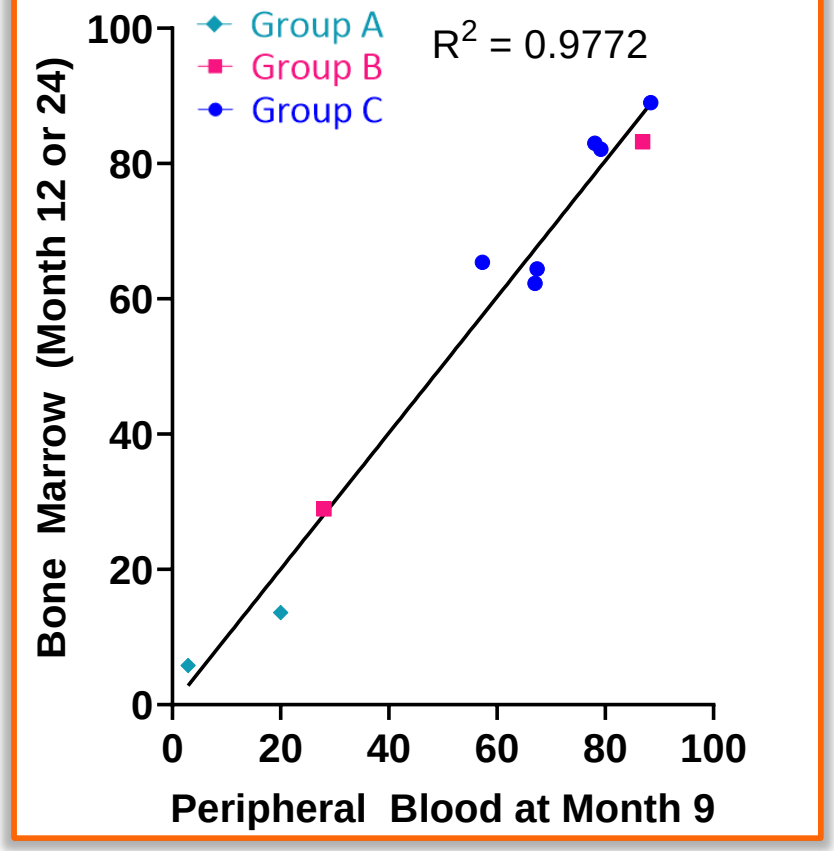
# Refined protocol results in higher %LVV+ in peripheral blood and LVV+ cells demonstrate stable engraftment



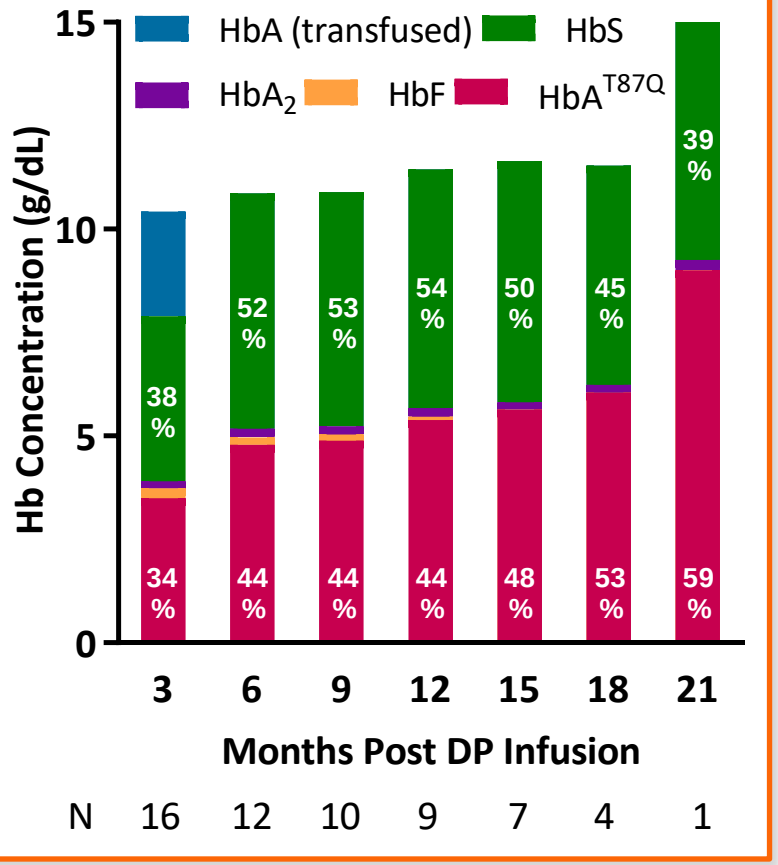

Each patient (n=6 Group A, n=2 Group B and n=17 Group C) is represented by a unique symbol or a unique combination of colored line and symbol
BFU-E, burst forming unit-erythroid; DP, drug product; LVV, lentiviral vector

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

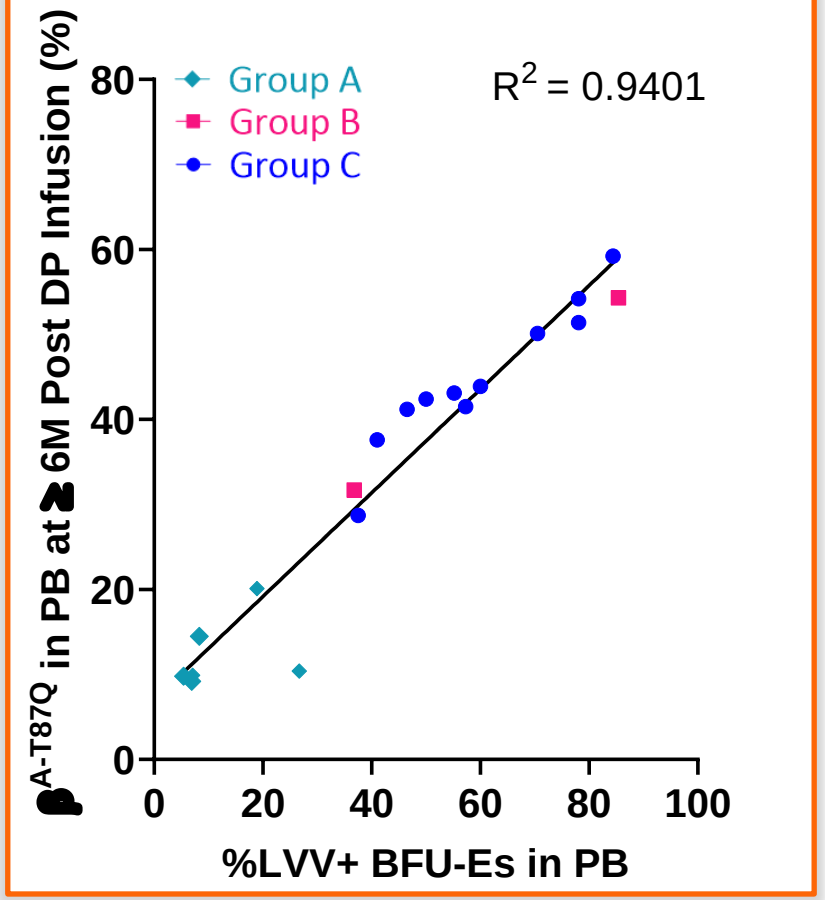
## %LVV+ in PB is a good proxy for BM LVV-marking




## Median Hb fractions in PB of Group C patients over time




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

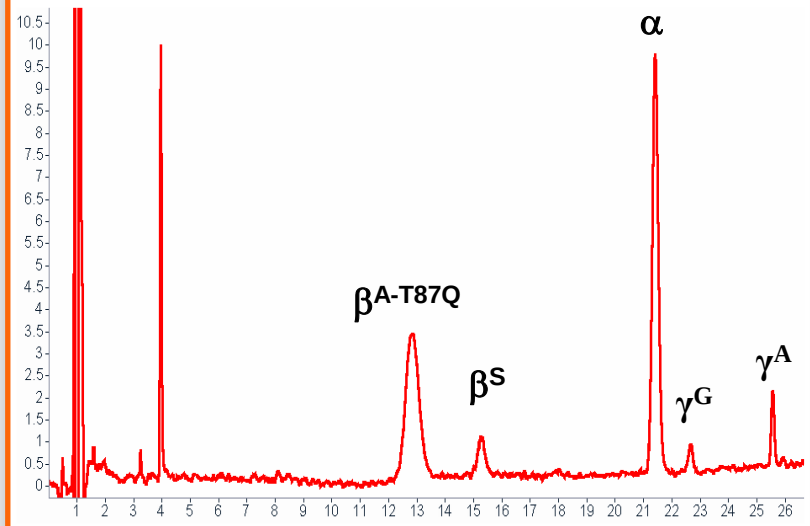



BFU-E, burst forming unit-erythroid; BM, bone marrow; DP, drug product; Hb, hemoglobin; LVV, lentiviral vector; PB, peripheral blood

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

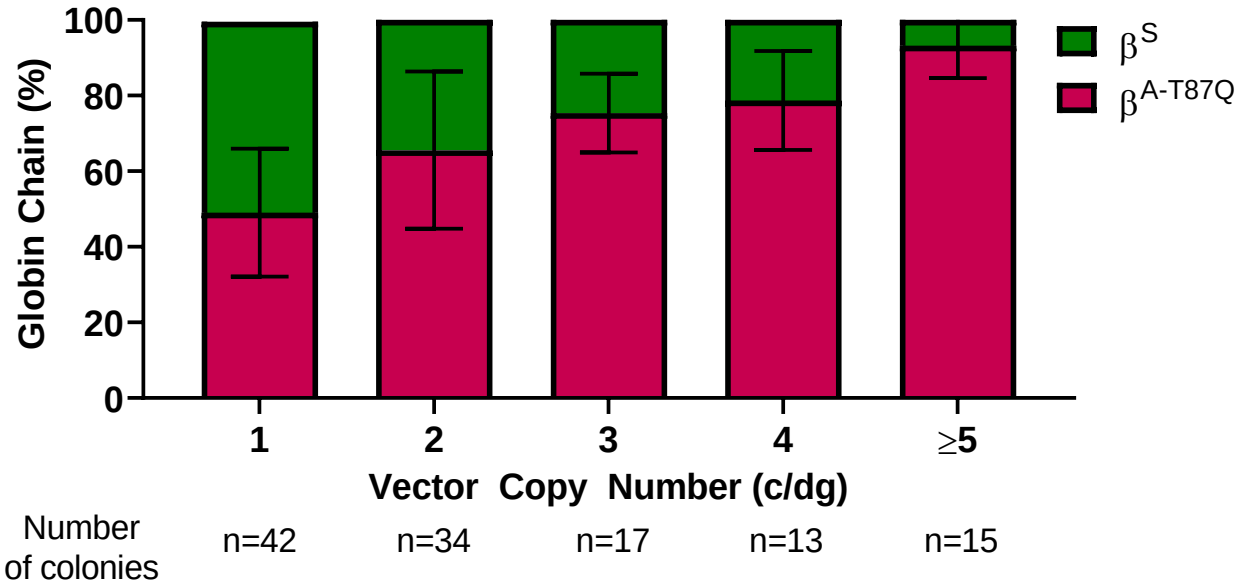
## Sample ratio of all β-globin chains

***Example for VCN = 3***




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

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



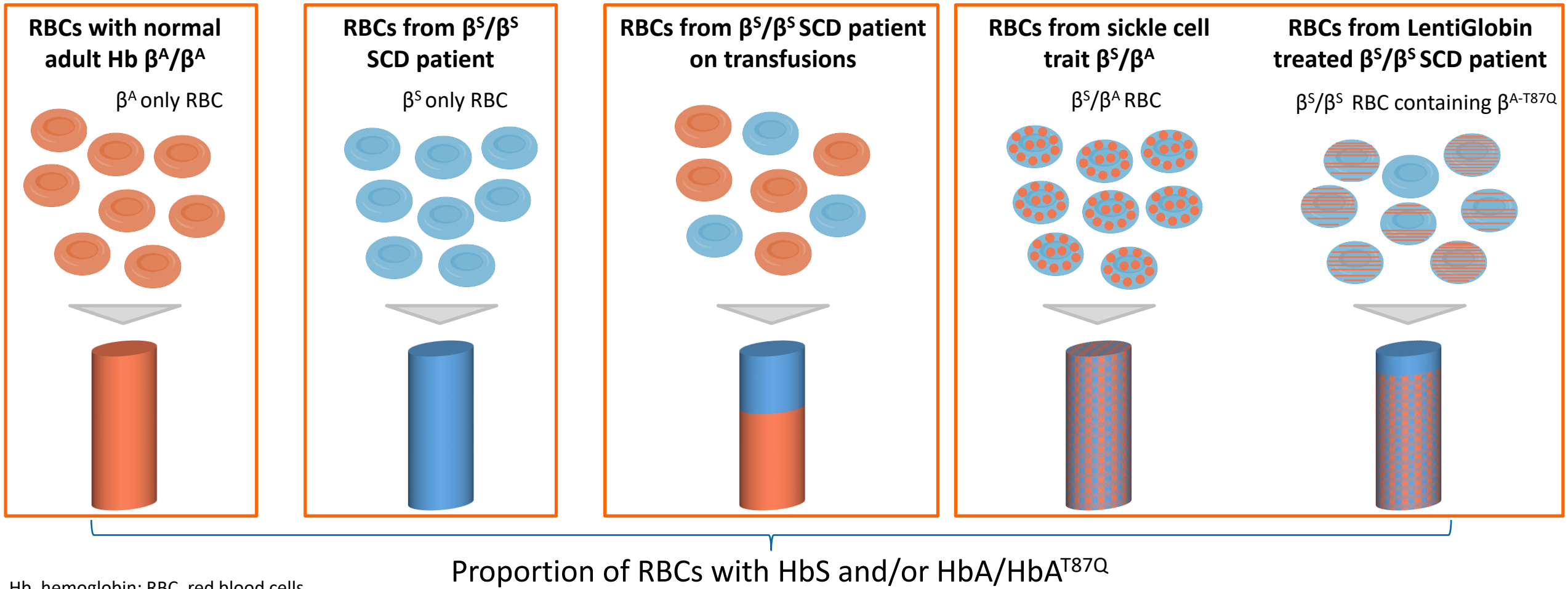

- $HbA^{T87Q}$ levels in these three patients at the time of analysis post DP infusion were 3.6 – 8.8 g/dL

- 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

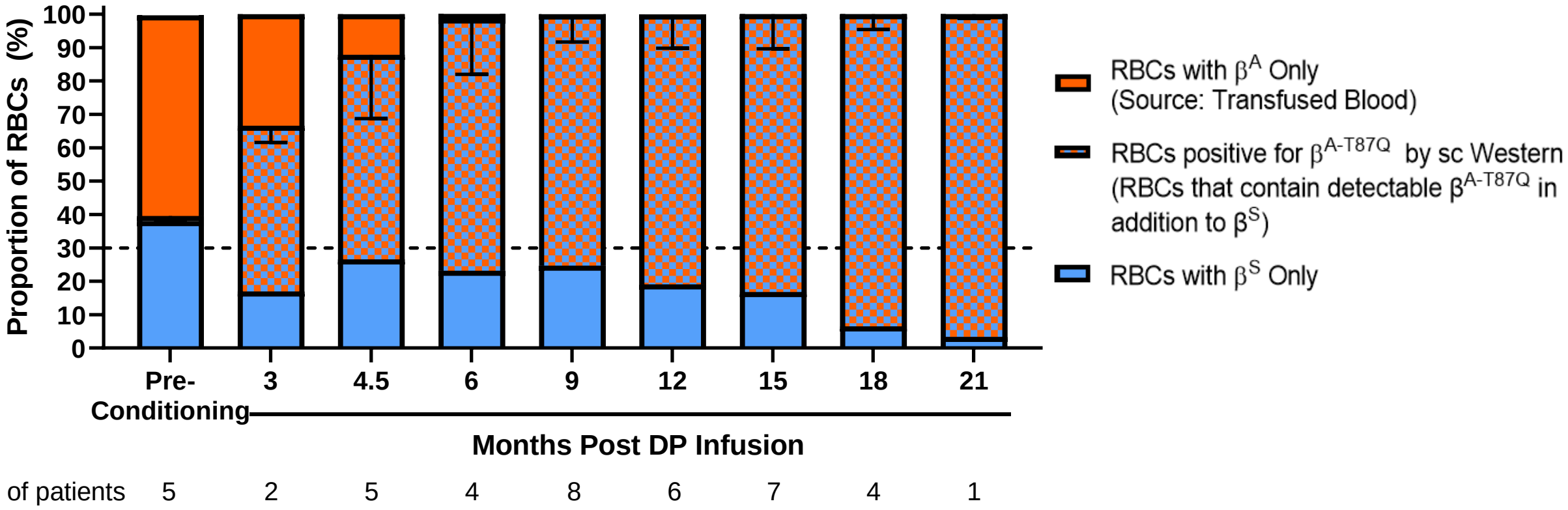
- Single red blood cell western with anti-$\beta^S$ or anti-$\beta^A/\beta^{A\text{-}T87Q}$ antibodies




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

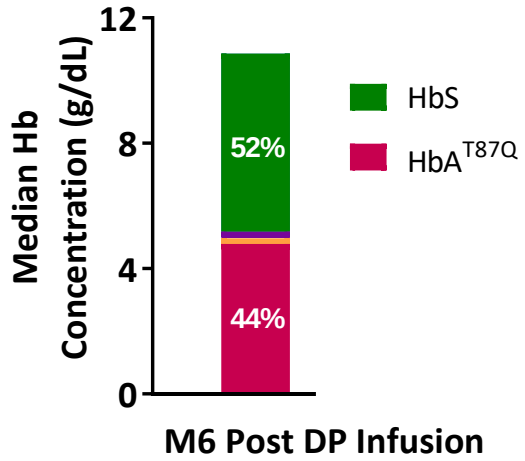



Mean ± SD is depicted - if N=1, data show technical replicates; *Pre-conditioning sample does not contain any $\beta^{A\text{-}T87Q}$, signal is due to error rate of multiples

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

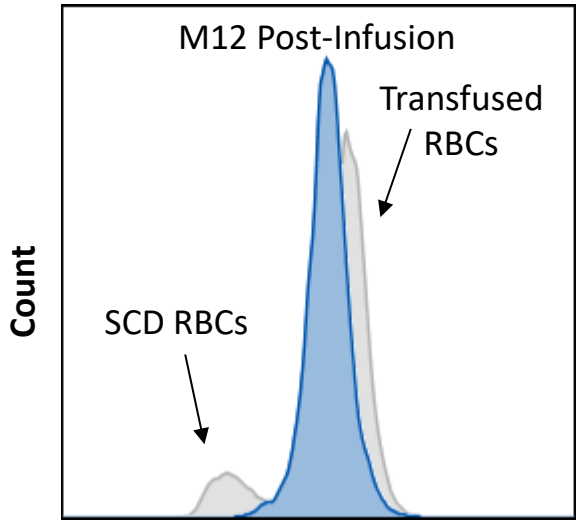
# $HbA^{T87Q}$ levels in RBCs from patients post DP infusion are estimated to be comparable to the amount of HbA per RBC in sickle cell trait

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

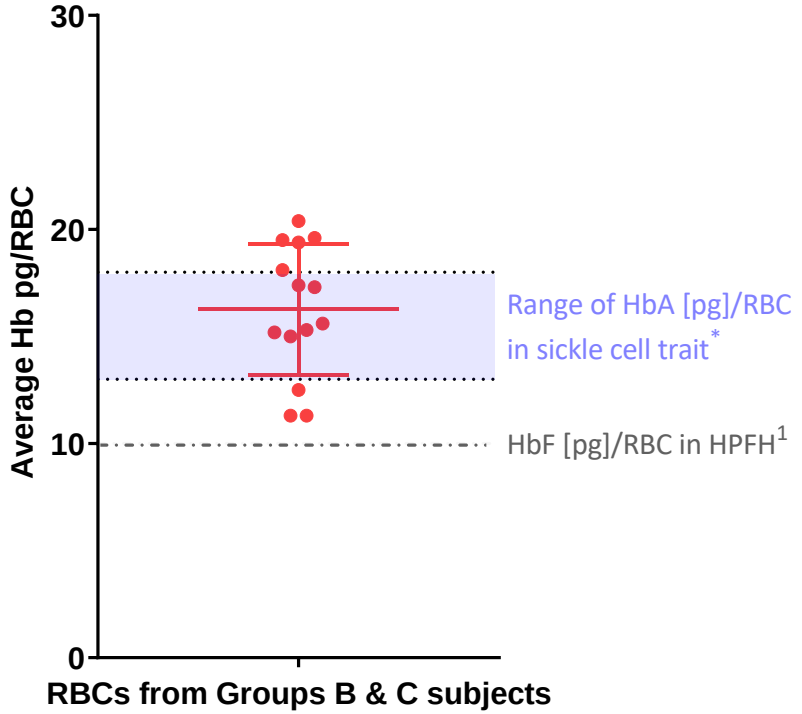



| Median MCH | |
|---|---|
| Baseline (n=30) | M6 post DP infusion (n=12) |
| 31.6 pg | 31.6 pg |

## 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

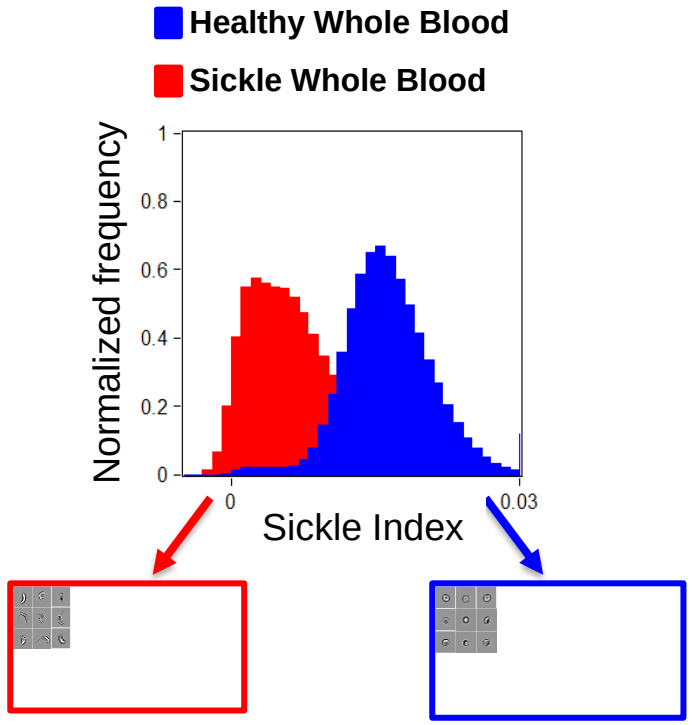
*Calculated using 50% HbA/RBC for the lower end of the range and 60% HbA/RBC for the upper end of the range

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

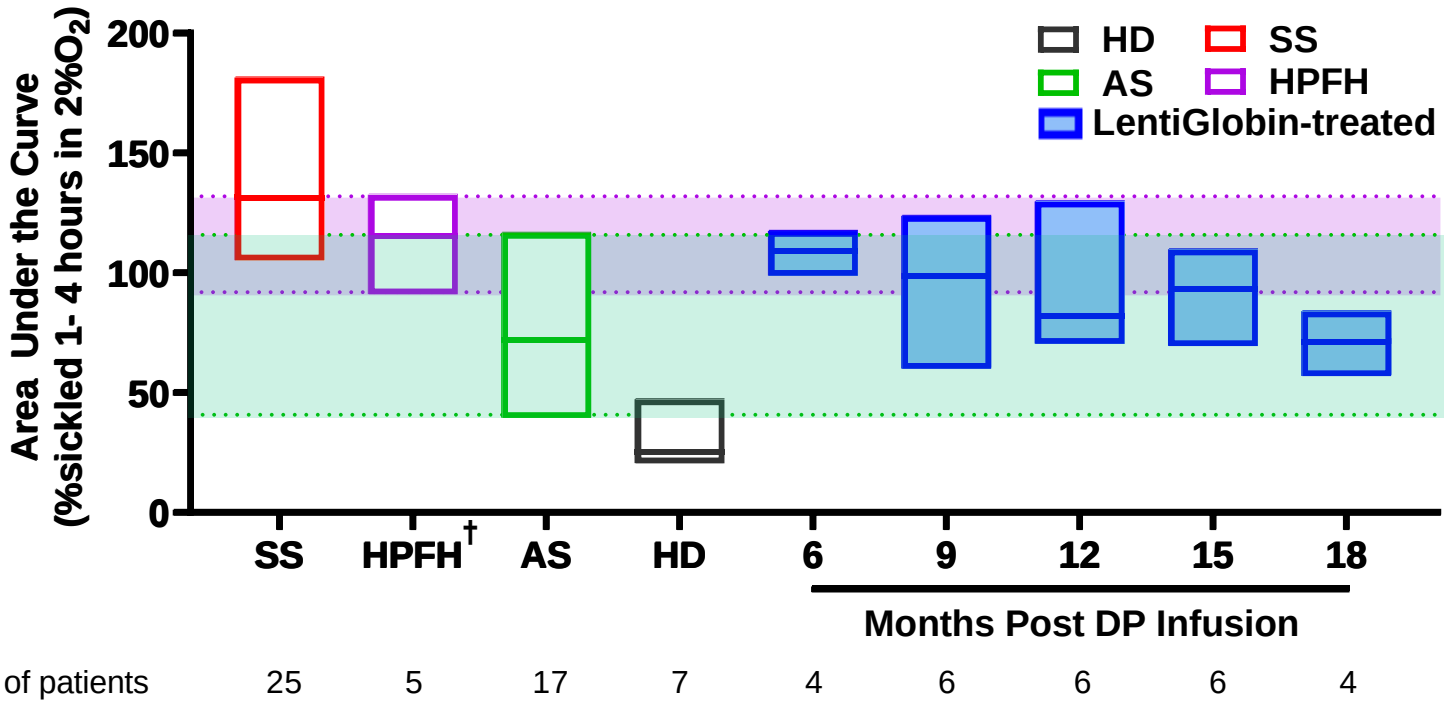
DP, drug product; Hb, hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; MCH, mean corpuscular hemoglobin; RBC, red blood cells

# Impact of intracellular $\beta^{A\text{-}T87Q}$ and $\beta^{S}$ 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 $\beta^{S}$ 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

**Tomorrow: May 15 at 8:15-8:30 am in the Clinical Trials Spotlight Symposium session**

| | 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 #1298**

**bluebird bio, Inc.,** Cambridge:
Jean-Antoine Ribeil
Sunita Goyal
Alex Miller
Wenmei Huang
Purvi Mody
Iva Kronja