# lovo-cel (bb1111) Gene Therapy for Sickle Cell Disease: Updated Clinical Results and Investigations into Two Cases of Anemia from Group C of the Phase 1/2 HGB-206 Study

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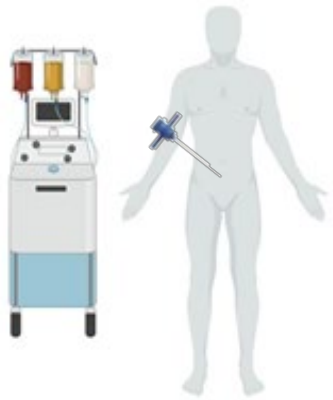
# HGB-206 study design: Group C

**≥60 days of pRBC transfusions to reduce stress erythropoiesis**

**HSPC collection**
N=43

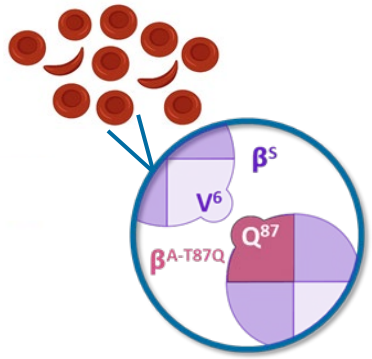


**Busulfan myeloablative conditioning**
N=36

**DP infusion**
N=36

DP manufactured at a centralized location

**Transduced HSPCs engraft and contribute to reconstitution of functional RBCs**




Modified RBCs express gene therapy-derived $HbA^{T87Q}$

**2-year follow-up**

**Long-term follow-up study (LTF-307)**

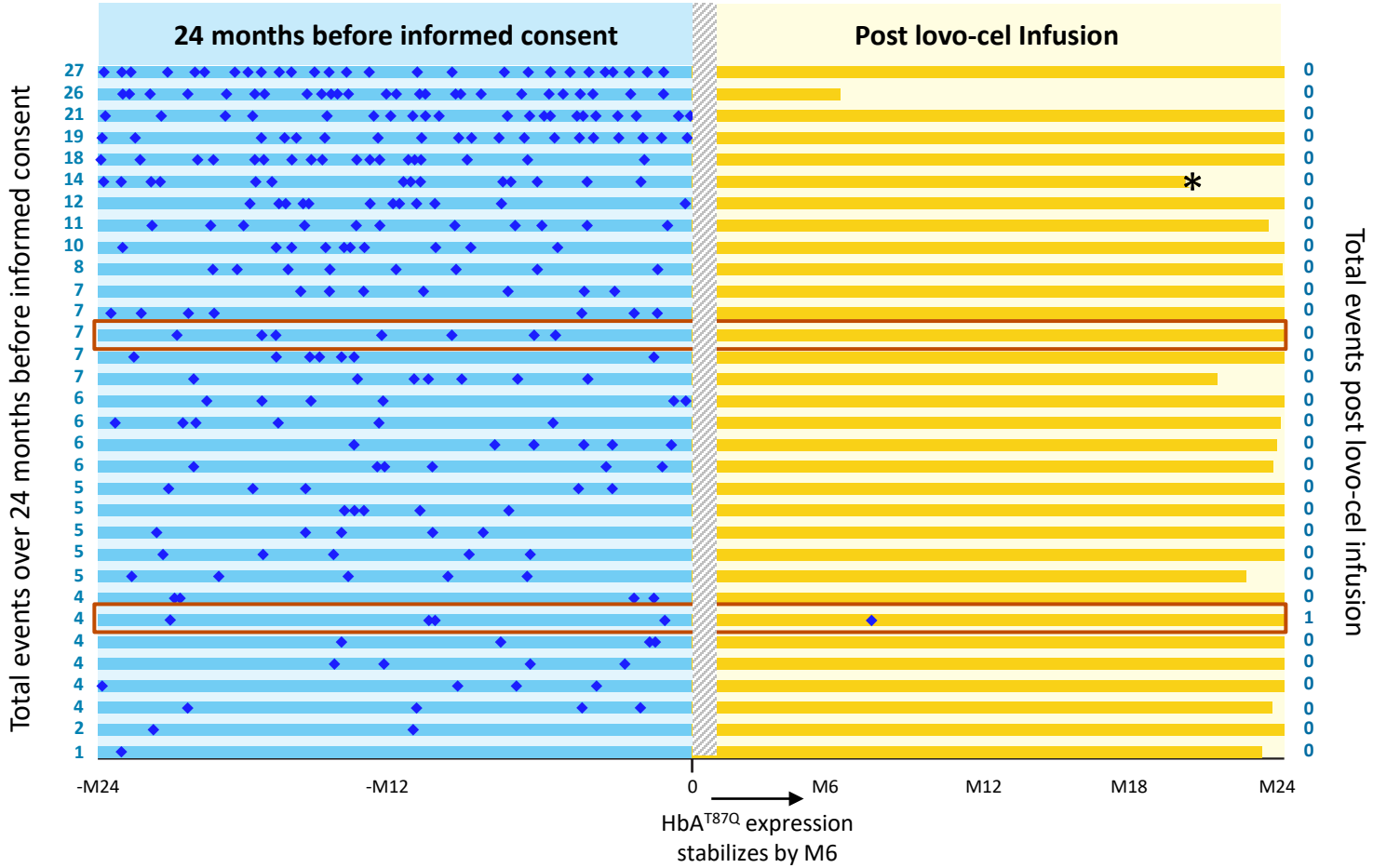
## *Inclusion criteria*

- Age ≥ 12 – ≤ 50 years at time of consent
- Diagnosis of SCD, with either $\beta^S/\beta^S$, $\beta^S/\beta^0$, or $\beta^S/\beta^+$ genotype
- ≥ 4 severe VOEs in the 24 months prior to informed consent
- Hydroxyurea failure or intolerance
- Karnofsky (≥ 16 years of age) or Lansky (< 16 years of age) performance status ≥ 60
- Treated and followed for ≥ 24 months prior to informed consent in center(s) that maintained detailed records on SCD history

**1.** Kanter et al. *NEJM.* 2022. **2.** Thompson et al. ASH 2020.

DP, drug product; Hb, hemoglobin; HSPC, hematopoietic stem and progenitor cells; pRBC, packed red blood cells; RBC, red blood cell; SCD, sickle cell disease; VOE, vaso-occlusive event.

# HGB-206 Group C: severe VOEs pre and post lovo-cel infusion




Protocol severe VOEs are shown.

Hb, hemoglobin; M, month; max, maximum; min, minimum; VOE, vaso-occlusive event.

# HGB-206 Group C: Safety profile

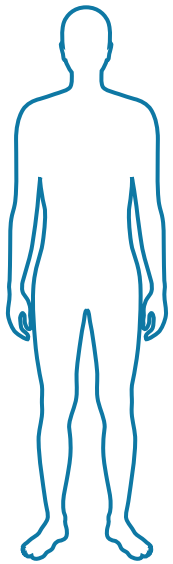
| **Serious treatment-emergent AEs**<br>*Reported in ≥2 patients* | **N=36**<br>n (%) |
|---|---|
| Pain | 4 (11.1) |
| Abdominal pain | 2 (5.6) |
| Anemia | 2 (5.6) |
| Drug withdrawal syndrome | 2 (5.6) |
| Nausea | 2 (5.6) |
| Suicidal ideation | 2 (5.6) |
| Vomiting | 2 (5.6) |

| **Grade ≥3 treatment-emergent AEs**<br>Reported in ≥3 patients | **N=36**<br>n (%) |
|---|---|
| Stomatitis | 25 (69.4) |
| Thrombocytopenia | 24 (66.7) |
| Neutropenia | 20 (55.6) |
| Febrile neutropenia | 15 (41.7) |
| Anemia | 14 (38.9) |
| Leukopenia | 12 (33.3) |
| Increased AST | 6 (16.7) |
| Increased GGT | 5 (13.9) |
| Nausea | 4 (11.1) |
| Increased ALT | 4 (11.1) |
| Decreased appetite | 4 (11.1) |
| Pain | 3 (8.3) |

- No cases of veno-occlusive liver disease
- No cases of graft failure or vector-mediated RCL
- No insertional oncogenesis seen in any lovo-cel treated patient to date
- As previously reported, patient with significant baseline SCD-related cardiopulmonary disease died >18 months post-infusion (considered unlikely to be related to lovo-cel)
- **Two patients had persistent anemia, erythroid dysplasia and low-level trisomy 8 in the bone marrow, prompting further investigation**

AE, adverse event; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; RCL, replication competent lentivirus; SCD, sickle cell disease.

# Adult patient with persistent anemia



**20-year-old female with $\beta^S/\beta^S$ and α-thalassemia trait ($-\alpha^{3.7}/-\alpha^{3.7}$)**

- History of VOEs and migraines, and had been prescribed hydroxyurea
- **No recent history of regular transfusions**
- **Erythroid dysplasia present at ~3% in baseline BM**

**Feb 2021**

Investigations into cause of anemia: BMA performed

- **No increased blast population**
- **10-20% dysplasia restricted to erythroid lineage**
  - Included nuclear irregularities such as binucleation and nuclear budding
- **Trisomy 8 detected using FISH in 6% of cells**
- **Karyotype was normal**
- Experienced sVOE coinciding with acute gastroenteritis

Initial Diagnosis: MDS

// *Not to scale*

**Jul 2020**
**lovo-cel infusion**

*DP characteristics within historical ranges*

**Post-treatment severe anemia** requiring chronic transfusions despite high PB VCN (4.6 c/dg at M6) and high percentage of $\beta^{A\text{-}T87Q}$ (49.0% at M6)

**Mar 2021 repeat BMA**

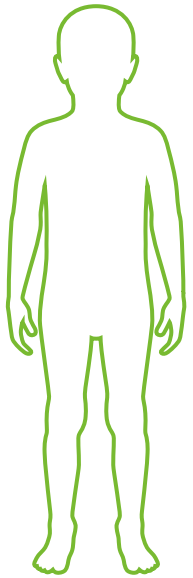
- **FISH normal**
- **Karyotype normal**
- **Continued erythroid dysplasia present but stable**

**Mar 2021**
**Revised diagnosis:**

**Transfusion-dependent anemia**

BM, bone marrow; BMA, bone marrow aspirate; DP, drug product; FISH, fluorescent *in situ* hybridization; M, month; MDS, myelodysplastic syndrome; PB, peripheral blood; sVOE, severe vaso-occlusive event; VCN, vector copy number; VOE, vaso-occlusive event.

# Pediatric patient with persistent anemia



**14-year-old male with $\beta^S/\beta^S$ and α-thalassemia trait ($-\alpha^{3.7}/-\alpha^{3.7}$)**

- History of chronic pain, silent stroke, iron overload, coronary artery dilation
- **History of hydroxyurea followed by chronic pRBC transfusions**
- **No baseline BM available**

**Jul 2021 (12M)**

**PB:**

- VCN was 3.32 c/dg
- HGB: 9.2 g/dL, ANC: 1600, PLT: 130x$10^3$/µL

**BM:**

- **No increased blast population**
- **Erythroid dysplasia in 5 -10% of cells**
- **Trisomy 8 detected by FISH in 7.7% of cells**
- **Karyotype normal**

**Diagnoses:**

Grade 1 anemia
Grade 2 neutropenia
Grade 1 thrombocytopenia

// *Not to scale*

**Jul 2020**
**lovo-cel infusion**

*DP characteristics within historical ranges*

**Oct/Nov 2021**

- Clinically well without pain
- HGB: 10 g/dL, ANC: 1860, PLT: 161x$10^3$/µL
- **Erythroid dysplasia in 10-20% of cells**
- **Karyotype normal**
- **Trisomy 8 detected by FISH in 5% of cells**

**Jul 2022**

- Clinically well without pain
- HGB: 9.7g/dL, ANC: 1830 , PLT: 267x$10^3$/µL
- **Erythroid dysplasia was 10-20%**
- **Karyotype normal**
- **Trisomy 8, 17 detected by FISH in 7.5% and 7% of cells, respectively**

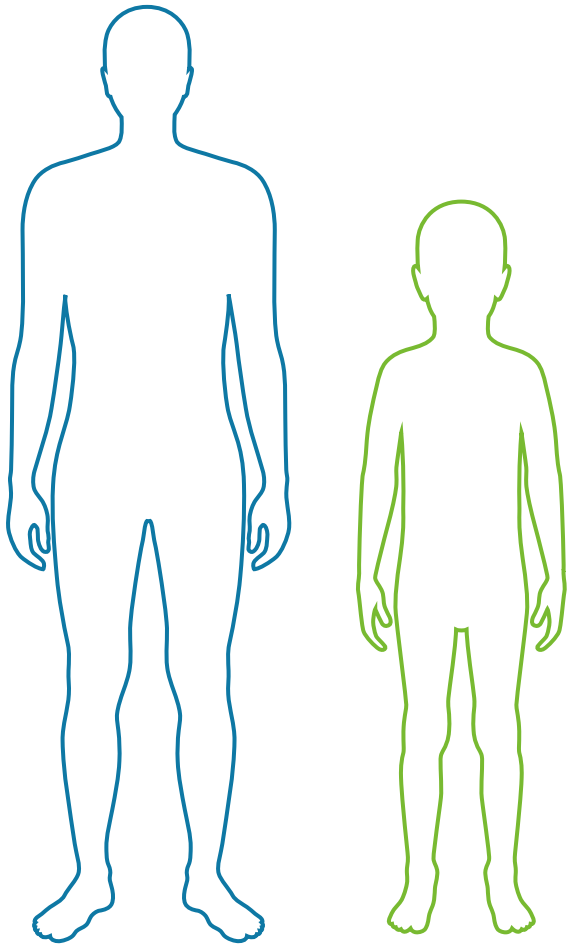
**Working diagnosis:**

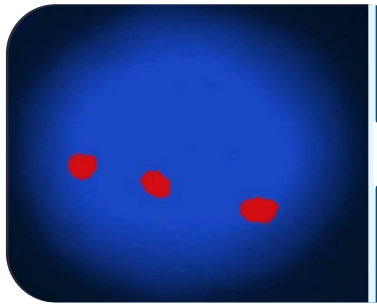
**Grade 1 anemia**

Normal range for ANC at site laboratory is 1800 – 5400.

ANC, absolute neutrophil count; BM, bone marrow; DP, drug product; FISH, fluorescent *in situ* hybridization; g/dL, grams per deciliter; HGB, hemoglobin; PB, peripheral blood; pRBC, packed red blood cells; PLT, platelets; VCN, vector copy number.

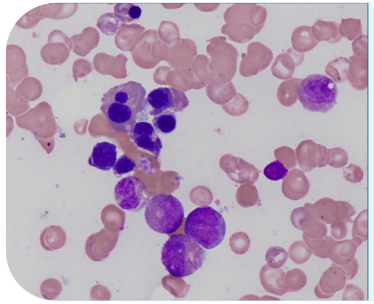
# Similar clinical symptoms and laboratory findings in 2 SCD patients after lovo-cel infusion





## Low-level trisomy 8 detected by FISH

- Unknown significance in non-malignant, gene therapy setting



## Erythroid dysplasia in bone marrow

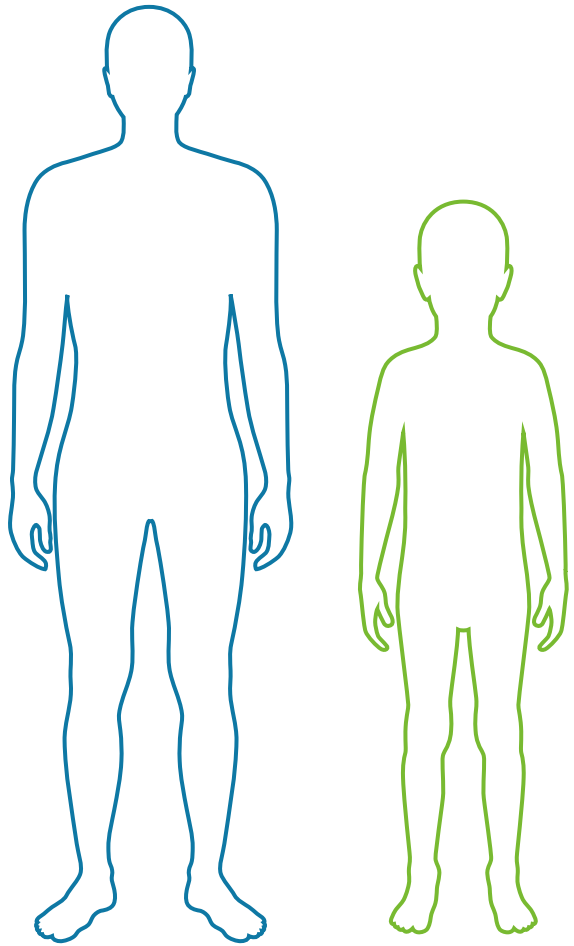
- Dysplasia only in the erythroid lineage and ≤20% in both patients
- No abnormal blast count

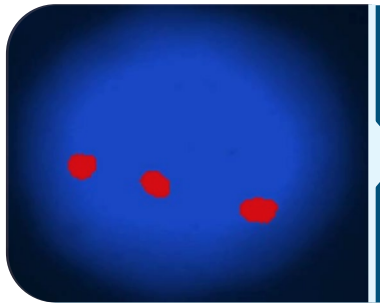
## Anemia with $\beta^{A\text{-}T87Q}$ levels > 40% in both patients

- Only patients with 2 α-globin gene deletions
- Adult (not pediatric) patient is transfusion dependent

FISH, fluorescent in situ hybridization; SCD, sickle cell disease.

# Similar clinical symptoms and laboratory findings in 2 SCD patients after lovo-cel infusion





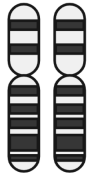
**Low-level trisomy 8 detected by FISH**

- **Unknown significance in non-malignant, gene therapy setting**

*Investigation to look for **emerging hematologic malignancy** – focusing on signs of a **clonal process** as hematologic malignancies are clonal*

FISH, fluorescent in situ hybridization; SCD, sickle cell disease.

# Methods used for investigation of malignant process



**Karyotype**

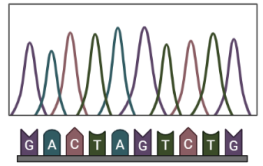
*"Gold Standard" for assessment of chromosomal abnormalities (i.e., aneuploidy and structural changes)*

**FISH**

*Cultured interphase nuclei are probed for specific chromosomal abnormalities*

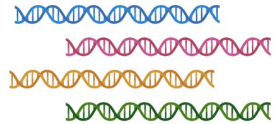
**ISA**

*Determines the location of vector insertions as well as relative contribution of each insertion to assess clonality of transduced cells*




**NGS: Rapid Heme Panel[1]**

*Sequencing of 88 genes associated with hematologic malignancy*



**Whole Exome Sequencing**

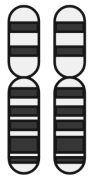
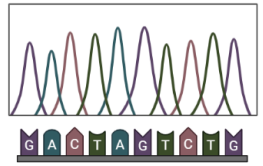
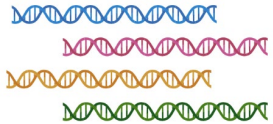
*Sequencing of all protein coding exons (~1% of genome)*

1. Kluk et al. *J Mol Diagn.* 2016.

FISH, fluorescent in situ hybridization; ISA, integration site analysis; NGS, next generation sequencing.

# No evidence of clonal process

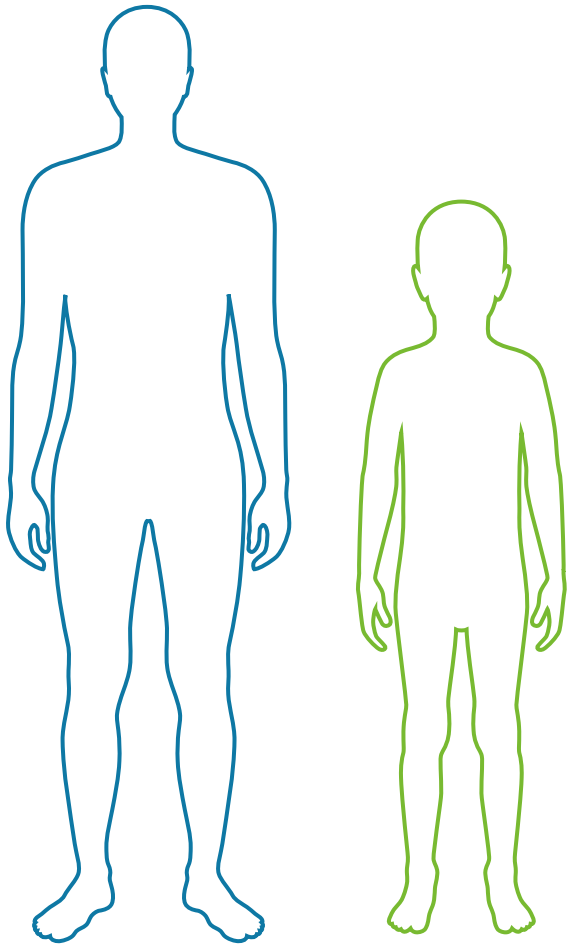
## *Unknown significance of FISH data in non-malignant, gene therapy setting*

| |  Karyotype |  FISH |  ISA |  NGS: Rapid Heme Panel |  Whole Exome Sequencing |
|---|---|---|---|---|---|
|  Adult | Normal in all BM samples M6–M18 post-infusion (n=4) | Transient low-level (6%) trisomy 8 in single BM sample (M6) | Highly polyclonal reconstitution<br>No clone > 0.43% in PB and 44,236 unique IS at M18 | No mutations detected except for four suspected germline VUS | Not Performed |
|  Pediatric | Normal in all BM samples M12–M24 post-infusion (n=3) | Low-level (5.0-7.7% ) trisomy 8 in all 3 BM samples (M12–M24)<br>Low-level (7.0%) trisomy 17 in M24 BM | Highly polyclonal reconstitution<br>No clone > 0.66% in PB or BM and >25,000 unique IS at M24 in PB and BM | No mutations detected except for one germline *TET2* VUS | No copy number changes detected in PB and BM |

1. Kluk et al. *J Mol Diagn.* 2016.

BM, bone marrow; FISH, fluorescent in situ hybridization; IS, integration site; ISA, integration site analysis; M, month; NGS, next generation sequencing; PB, peripheral blood; VUS, variant of unknown significance.

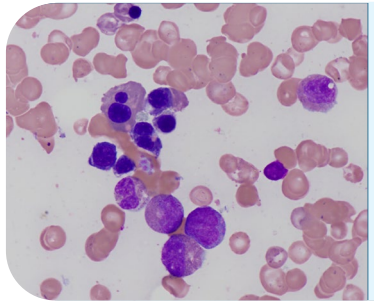
# Similar clinical symptoms and laboratory findings in 2 SCD patients after lovo-cel infusion





## Low-level Trisomy 8 detected by FISH

- Unknown significance in non-malignant, gene therapy setting



## Erythroid dysplasia in bone marrow

- Dysplasia only in the erythroid lineage and ≤20% in both patients
- No abnormal blast count



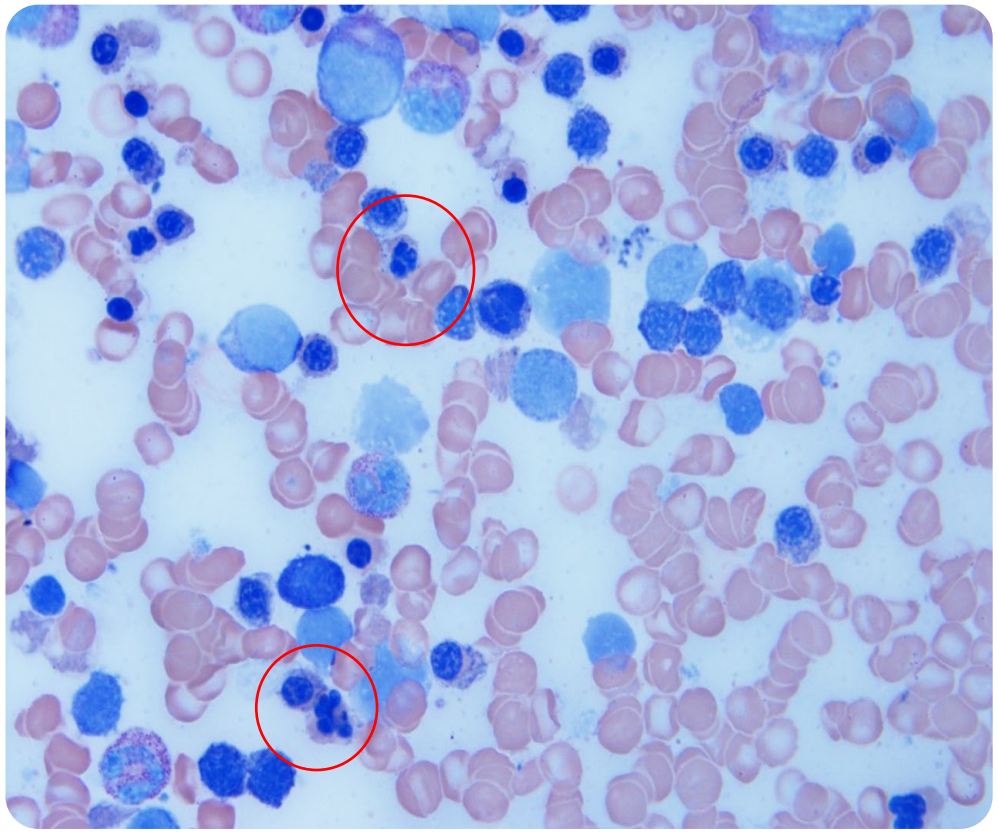
## Anemia with $\beta^{A\text{-}T87Q}$ levels > 40% in both patients

- Only patients with 2 α-globin gene deletions
- Adult (not pediatric) patient is transfusion dependent

FISH, fluorescent in situ hybridization; SCD, sickle cell disease.

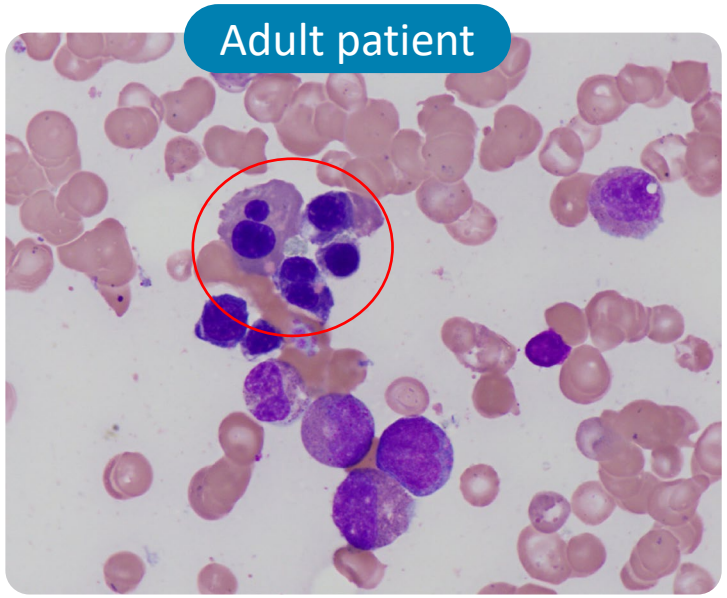
# Erythroid restricted dysplasia identified in the patients with two α-globin deletions is similar to dyserythropoiesis identified in TDT patients

## HGB-207 patient - baseline

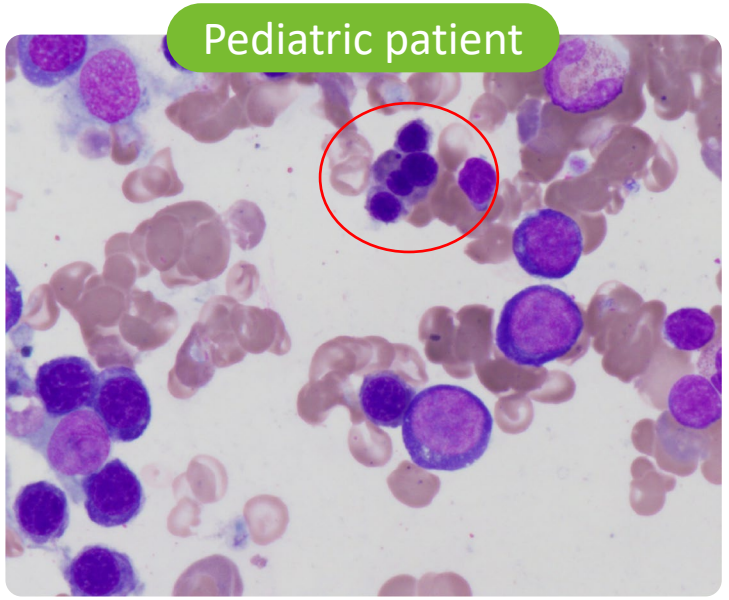


Nuclear abnormalities circled in red

- All TDT patients screened by bone marrow aspirate in HGB-207 (n = 22) had dyserythropoiesis
- Dyserythropoiesis described as nuclear abnormalities with nuclear budding, multinucleation, and nuclear karyorrhexis, as reported in the literature[1,2]




Month 6 visit

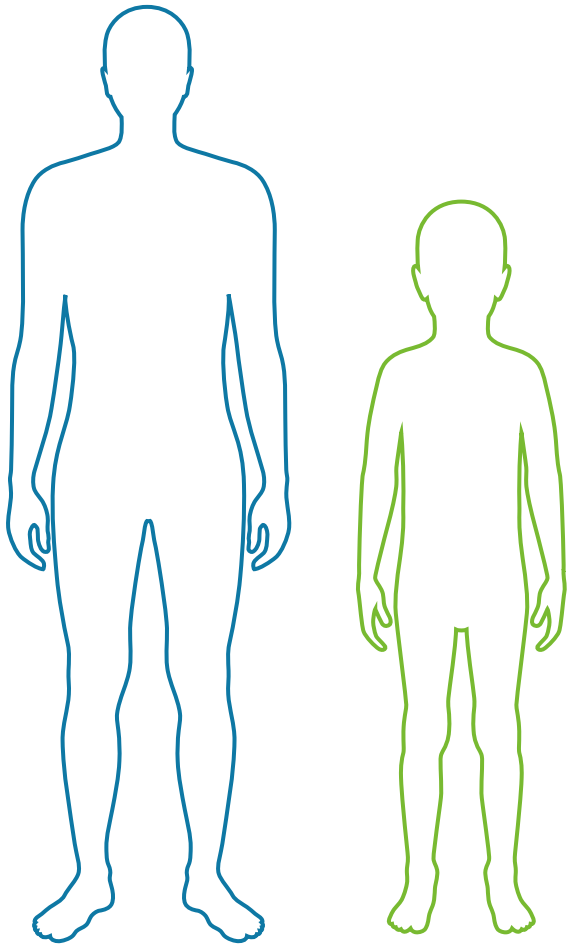



Unscheduled visit ~15 months post-transplant

**1.** Wickramasinghe et al. *Br J Haematol.* 1981. **2.** Austin et al. *Am J Hematol.* 2019

TDT, transfusion dependent thalassemia.

# Similar clinical symptoms and laboratory findings in 2 SCD patients after lovo-cel infusion





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- No abnormal blast count

## Anemia with $\beta^{A\text{-}T87Q}$ levels > 40% in both patients

- Only patients with 2 $\alpha$-globin gene deletions
- Adult (not pediatric) patient is transfusion dependent

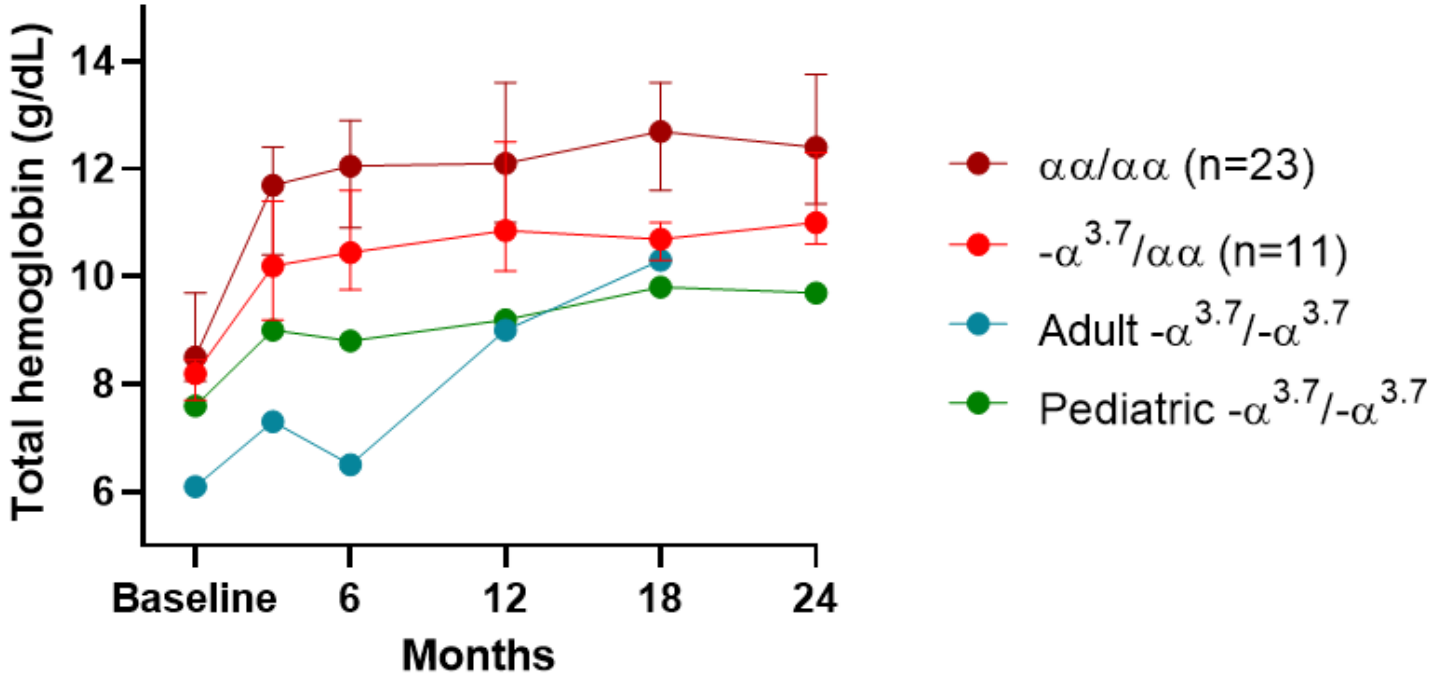
FISH, fluorescent in situ hybridization; SCD, sickle cell disease.

# The adult and pediatric patients with persistent anemia both had two α-globin deletions ($-\alpha^{3.7}/-\alpha^{3.7}$)

Only these two patients (5.6%) had two α-globin deletions and 11 patients (30.6%) had a single α-globin deletion

**Lower average hemoglobin was observed in Group C patients with single α-globin deletions**

Parallels reports of lower increase in total hemoglobin with hydroxyurea in adults[1] and children[2] with SCD and α-thalassemia trait

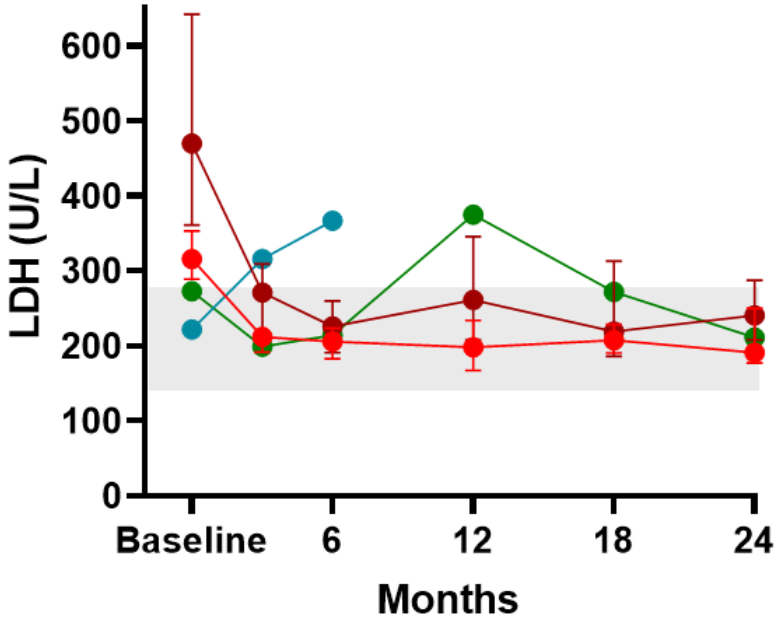


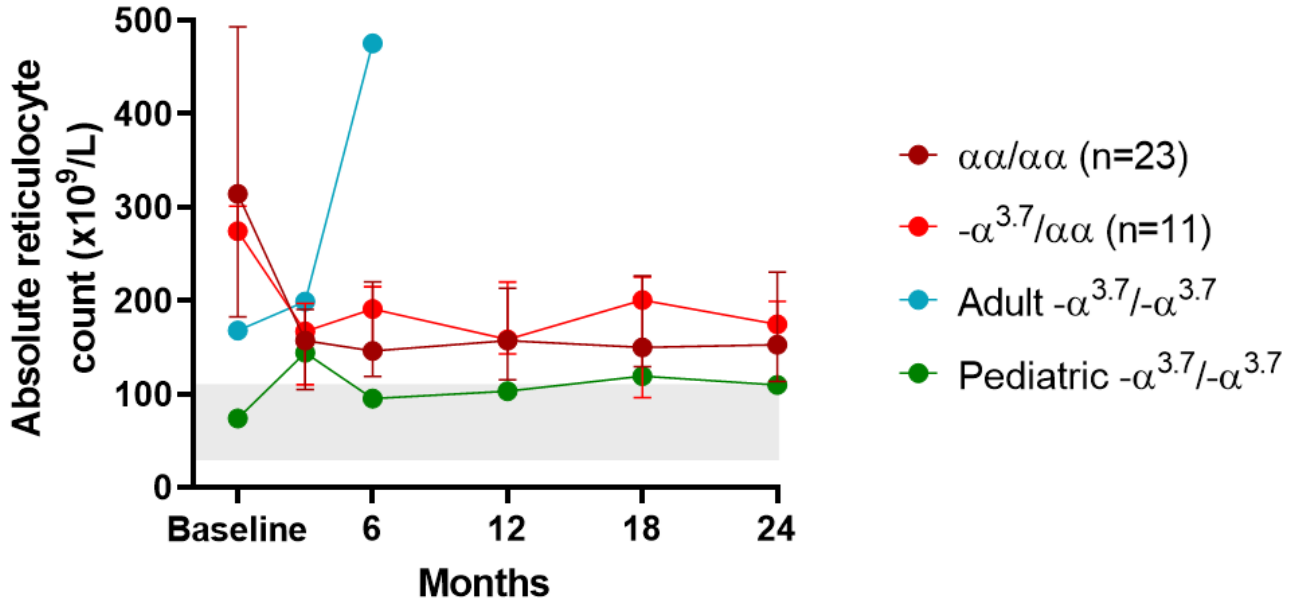

Total hemoglobin shown as Median (Q1, Q3).
Adult patient began receiving regular transfusions after their Month 6 CBC

**1.** Darbari et al. *Eur J Haematol.* 2013. **2.** Sheehan et al. *Am J Hematol.* 2013

# Alternative reasons for anemia ruled out

Pediatric patient's markers of hemolysis within range of other Group C patients.
Adult patient's markers align with BM and PB morphology and is consistent with an α-thalassemia phenotype.



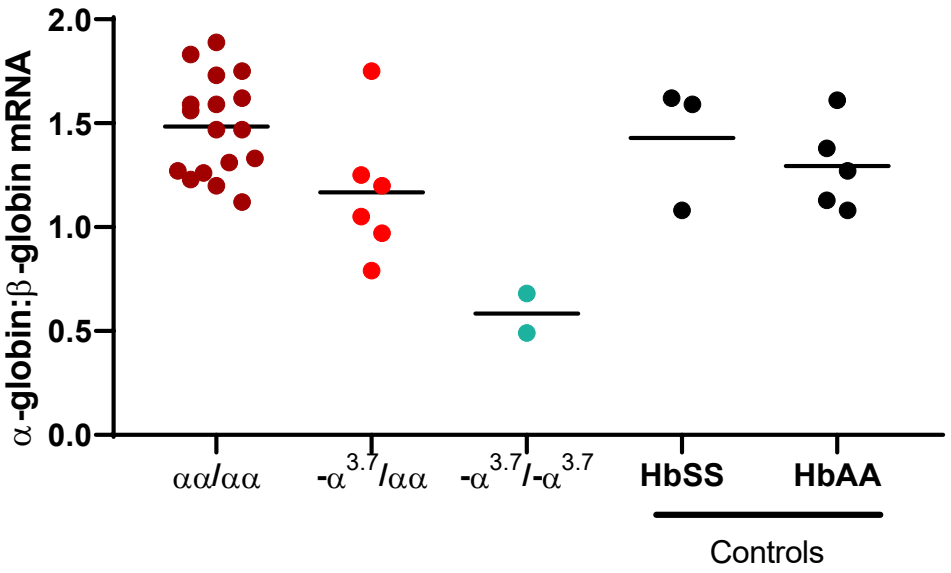

αα/αα and –α$^{3.7}$/αα values shown as Median (Q1, Q3). Laboratory assessments of adult patient not presented once regular transfusions were started
Follow up for one of the 11 –α$^{3.7}$/αα patients is ~6 months out from drug product infusion

- Anemia not associated with viral infection, immune-mediated hemolytic anemia, or vitamin deficiency
- Adult patient had increased orthochromatic normoblasts in BM (22% at Month 6) and a blood smear consistent with an α-thalassemia phenotype (i.e., target cells, polychromasia, etc.)

BM, bone marrow; LDH, lactate dehydrogenase; PB, peripheral blood; U/L, units per liter.

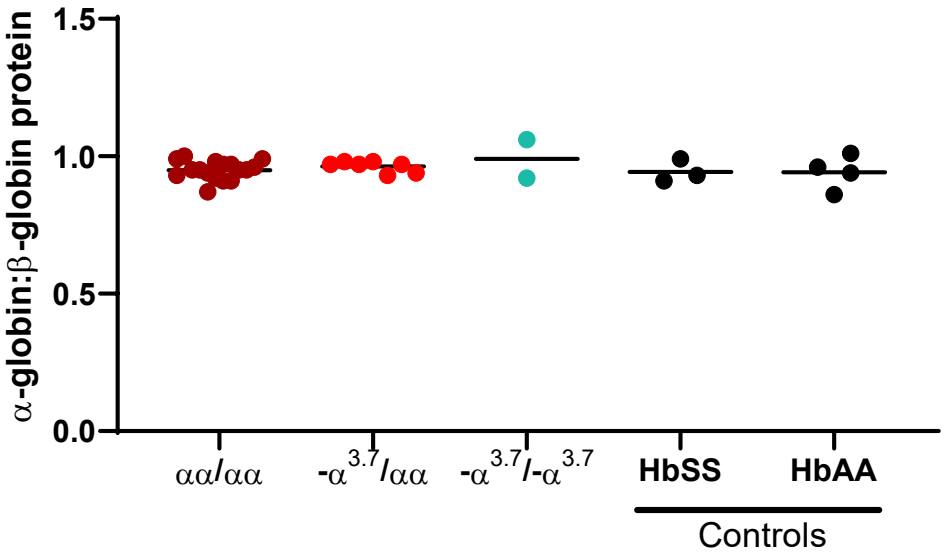
# Expected decreases in α:β-globin mRNA with α-globin gene deletions do not cause α:β-globin protein imbalance in PB

Reticulocytes with α-globin gene deletion(s) have expected decreased α:β-globin mRNA[1,2]




Suggests excess β-globin mRNA *and potentially protein* with α-globin gene deletion

Protein chain imbalance not observed in reticulocytes despite lower α:β-globin mRNA




HbH was observed intermittently and at very low levels, if at all, in PB[3,4]

**1.** Benz. *Am J Pediatr Hematol Oncol.* 1984. **2.** Chaisue et al. *Clin Biochem*. 2007. **3.** Thompson et al. *Am J Hematol.* 1989. **4.** Matthay et al. *J Clin Invest.* 1979.

Hb, hemoglobin; mRNA, messenger ribonucleic acid; PB, peripheral blood.

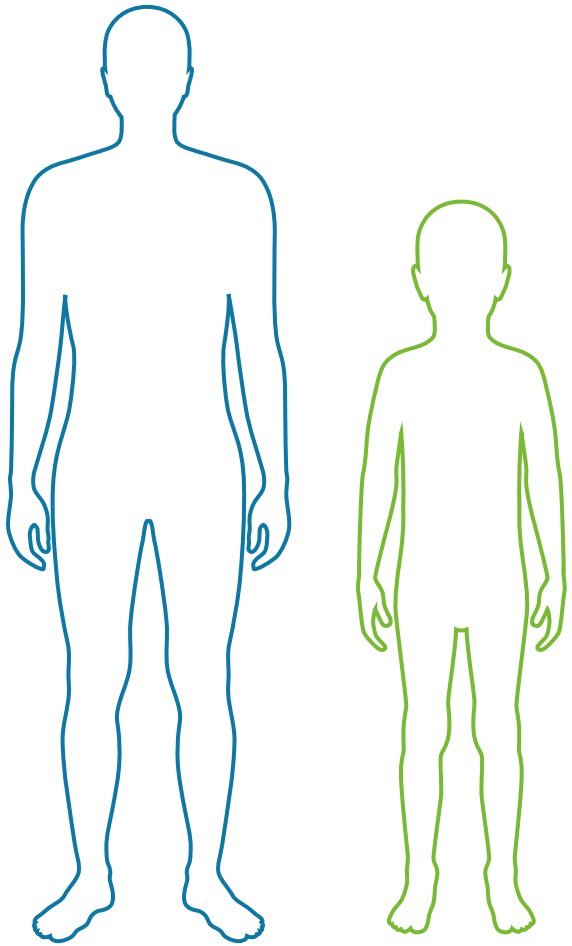
# Totality of evidence does not support current or emerging MDS diagnosis

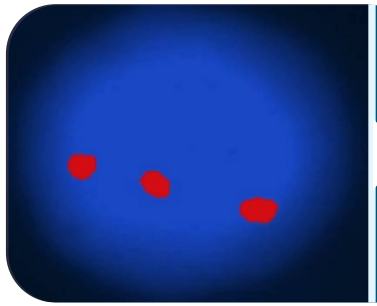
| Diagnostic and clinical features of MDS | Adult patient | Pediatric patient |
|---|---|---|
| **Clonality** | No clonal process identified (vector related or otherwise) | No clonal process identified (vector related or otherwise) |
| **Driver mutations consistent with MDS** | No mutations or aneuploidy identified by NGS, SNP microarray or karyotype<br>*FISH results normal after M6* | No mutations or aneuploidy identified by NGS, WES, or karyotype<br>*Low level trisomy 8 and 17 in BM only via FISH* |
| **Clinical symptoms** | Patient is now transfusion dependent and experiencing intermittent exacerbations of chronic pain | Patient is clinically well and has had no VOEs post-transplant |
| **Changes to blood counts** | No cytopenia aside from transfusion dependent anemia | Neutropenia resolved<br>Untransfused hemoglobin was 9.7 g/dL at M24 |
| **Dysplasia >10%** | Dysplasia is consistent with stress dyserythropoiesis | Dysplasia is consistent with stress dyserythropoiesis |

FISH, fluorescence *in situ* hybridization; g/dL, gram per deciliter; M, month; MDS, myelodysplastic syndrome; NGS, next generation sequencing; SNP, single nucleotide polymorphism; VOE, vaso-occlusive event; WES, whole exome sequencing.

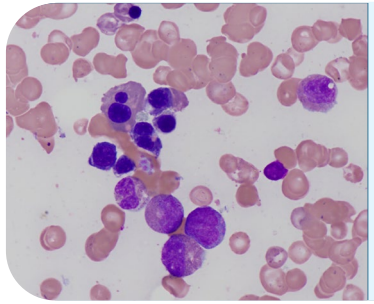
# Working diagnosis: α-thalassemia trait, possibly exacerbated by treatment with lovo-cel, likely driver of constellation of clinical and laboratory findings

*≥2 α-globin gene deletions added as exclusion criteria*





No evidence of clonal process; not consistent with emerging hematologic malignancy



Erythroid restricted dysplasia in bone marrow consistent with hemoglobinopathy

Anemia likely related to α:β-globin chain imbalance possibly exacerbated by treatment with lovo-cel

# Thank you to the study site members as well as the study participants and their families

**Ann and Robert H. Lurie Children's Hospital of Chicago, Northwestern University**

- Peter Chase

**Medical University of South Carolina**

- Brandi Day
- Michelle Hudspeth
- Jennifer Jaroscak

**Children's Hospital of Philadelphia**

- Janet Kwiatkowski
- Alexis Thompson
- Pranaya Venkatapuram

**UCSF Benioff Children's Hospital**

- Mark Walters
- Cyrus Bascon
- Marci Moriarty
- Frans Kuypers

**Yale New Haven Children's Hospital**

- Lakshmanan Krishnamurti

**Children's Healthcare of Atlanta**

- Suhag Parikh
- Lilian Okparaocha
- Megan Hanby

**Hackensack University Medical Center**

- Stacey Rifkin-Zenenberg
- Elana Smilow

**Cohen Children's Medical Center**

- Banu Aygun
- Alicha Paul
- Judene Mavrikis

**National Institutes of Health, Molecular and Clinical Hematology Branch**

- John Tisdale
- Matt Hsieh
- Naoya Uchida
- Stephanie Helwing
- Rick Gustafson
- Wynona Coles

**Columbia University Medical Center**

- Markus Mapara
- Monica Bhatia
- Beatriz Raposo Corradini
- Matt Chiaramonte

**University of North Carolina**

- Kimberly Kasow
- Catherine Cheng

**University of Alabama**

- Julie Kanter
- Michele Blue

**ProtaGene GmbH**

- Ivan Labik

**Independent reads of bone marrow**

- Robert Hasserjian
- Shunyou Gong

**Advisors**

- John DiPersio
- Coleman Lindsley

**bluebird bio, Inc.**

- Brandi Blount
- David Monteiro
- Melissa Bonner
- Heidi Elliot
- Lauryn Christiansen
- Qiao Li
- McKinley Nickerson
- Manisha Pradhananga
- Alex Miller
- Ketaki Kadam
- Alexandria Petrusich
- Teresa Curto