Polyclonality Strongly Correlates with Biological Outcomes and Is Significantly Increased Following Improvements to the Phase 1/2 HGB-206 Protocol and Manufacturing of LentiGlobin for Sickle Cell Disease (SCD; bb1111) Gene Therapy (GT)

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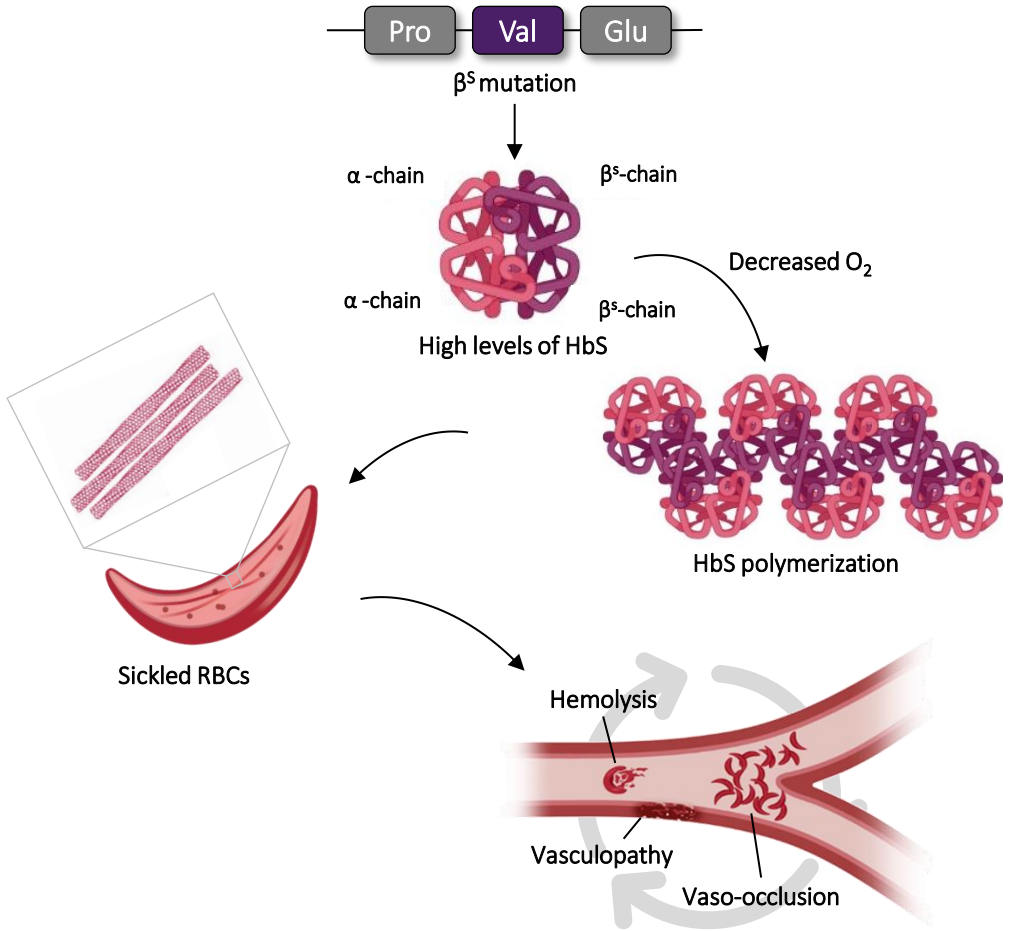
Disclosure

- John F Tisdale
  - Nothing to disclose

SCD is characterized by high morbidity and early mortality

Pathophysiology of SCD[1]



Complications[2,3]

- Chronic daily pain
- Vaso-occlusive pain
- Anemia
- Cerebral vasculopathy/stroke
- Retinopathy
- Acute chest syndrome
- Pulmonary hypertension
- Hepato-splenic sequestration
- Cardiovascular complications
- Priapism
- Kidney disease
- Sudden death
- Leg ulcers
- Organ failure
- Osteonecrosis

Glu, glutamic acid; HbS, sickle hemoglobin; Pro, proline; RBC, red blood cell; SCD, sickle cell disease; Val, valine.

1. Kato GJ, et al. *Nat Rev Dis Primer*. 2018;4:18010; 2. Hassell K., *Am J Prev Med.* 2010;38(Supplement 4):S512–21; 3. Kanter, et al. *Blood Rev*. 2013;27(6):279- 287.

HGB-206*: Study design

HSPC collection

Bone marrow harvest or mobilization with plerixafor & apheresis

Busulfan myeloablative conditioning

DP infusion

Transduced HSPCs engraft and contribute to reconstitution of functional RBCs

2-yr follow-up

Long-Term Follow-Up Study

LentiGlobin DP centralized manufacturing

Select CD34+ cells

Transduce with BB305 LVV

Cryopreserve, test, release DP

β^{T87Q} α α β^{T87Q}

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

*NCT02140554.

DP, drug product; HbA^{T87Q}, Hb with modified β-globin gene ($\beta^{A\text{-}T87Q}$); HSPC, hematopoietic stem and progenitor cell; LVV, lentiviral vector; RBC, red blood cell.

Protocol evolution in the open-label, phase 1/2 HGB-206 study of LentiGlobin for SCD (bb1111)

HSPC Source Shift

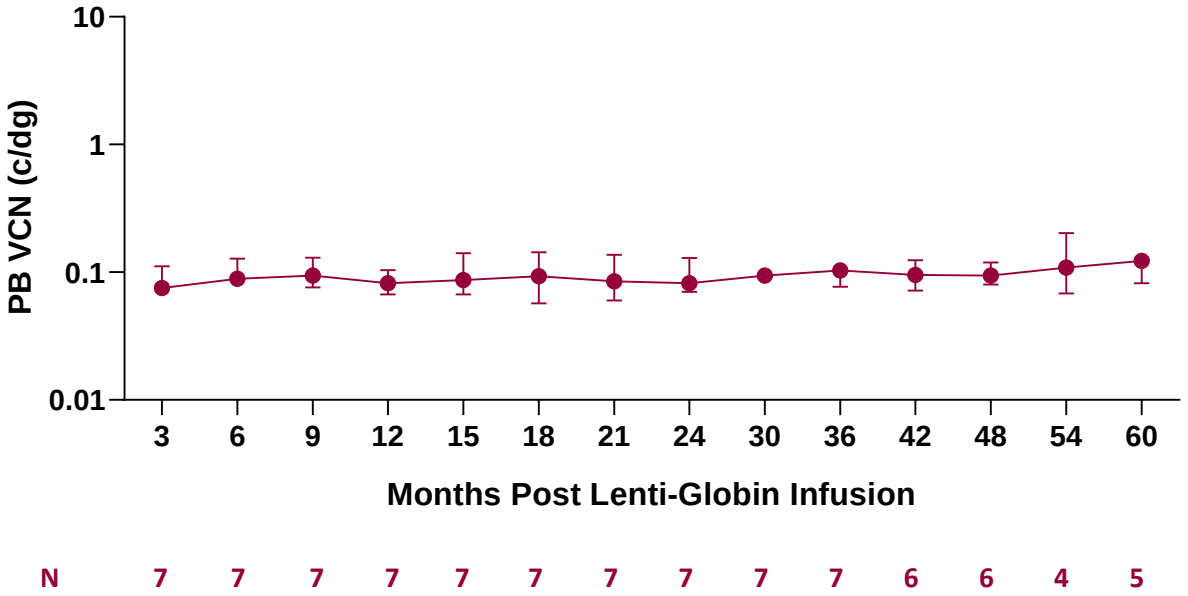
	Group A[1-6] n=7	Group B[1-6] n=2	Group C[1-6] n=35
Pre-collection transfusion regimen	Optional	Required	Required
HSC Source	Bone marrow	Bone marrow	Plerixafor-mobilization and apheresis
Conditioning AUC Target, µmol*min per dose†	4,000–4,500 (median AUC: 4747)	5000 (median AUC: 5136)	5,000 (median AUC: 4829)
Manufacturing Process	Original	Original → Refined	Refined
Total Cell Dose, x10^6 cells/kg (Median total CD34+, CD34hi/+ HSPCs)	Low (2.1, 1.6)	Medium (2.7, NA)	High‡ (6.9, 5.7)
Transduction Efficiency (Median DP VCN, Median % Transduced)	Low (0.7, 27.7%)	High (3.1, 77.4%)	High (3.7, 80.3%)

†For a once daily dosing regimen[5,6]; ‡following transduction of isolated CD34+ HSPCs with BB305 LVV, the bb1111 cell dose will be ≥3.0×10^6 CD34+ cells/kg for each patient[3].

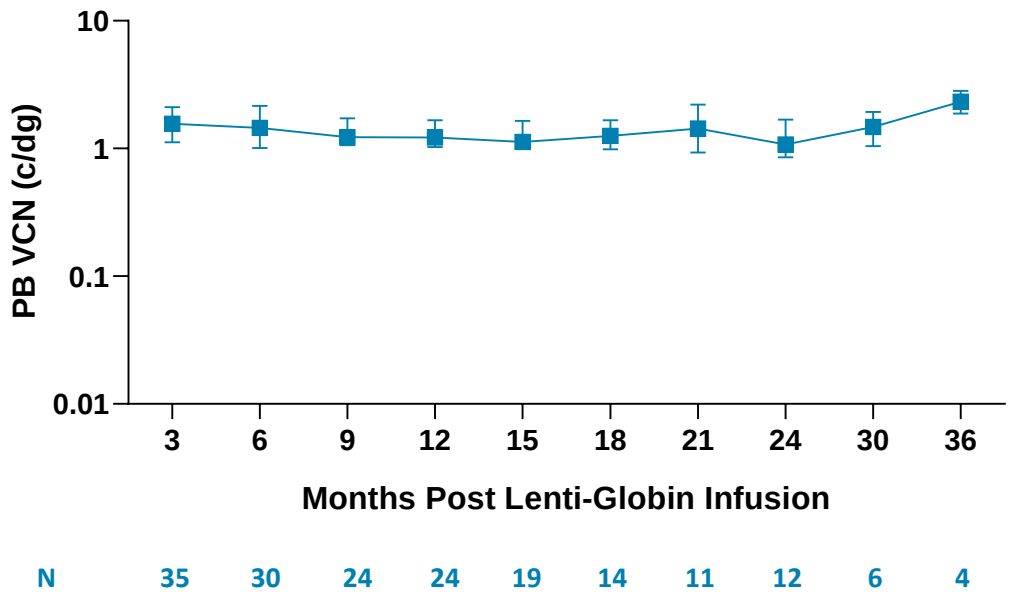
AUC, area under the curve; DP, drug product; GT, gene therapy; HSPC, hematopoietic stem and progenitor cell; VCN, vector copy number. 1. bluebird bio data on file. 2. Kanter J, et al. *Blood*. 2017;130 (Supplement 1):527. 3. Kanter J, et al. Blood. 2018; 132 (Supplement 1): 1080. 4. Kanter J. EHA *Library*. 2020; Abstract #S282. 5. Thompson AA, et al. ASH 2020. Abstract #677. 6. Tisdale JF, et al. *Am J Hematol*. 2020;95(9):E239-E242.

HGB-206 Groups A and C: PB VCN stabilizes by Month 6 post-LentiGlobin infusion and is sustained throughout follow-up

Group A



Group C

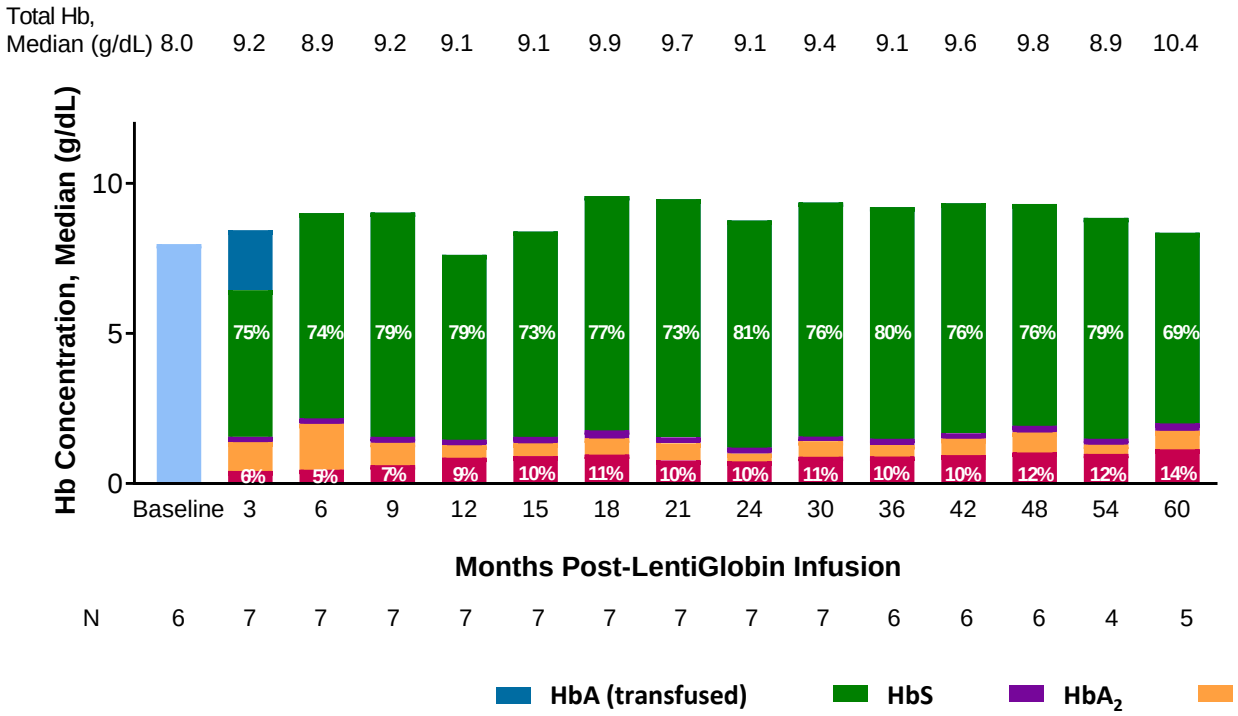


- Median PB VCN was higher in Group C than in Group A (1.45 c/dg vs 0.09 c/dg) at Month 6 post-LentiGlobin infusion

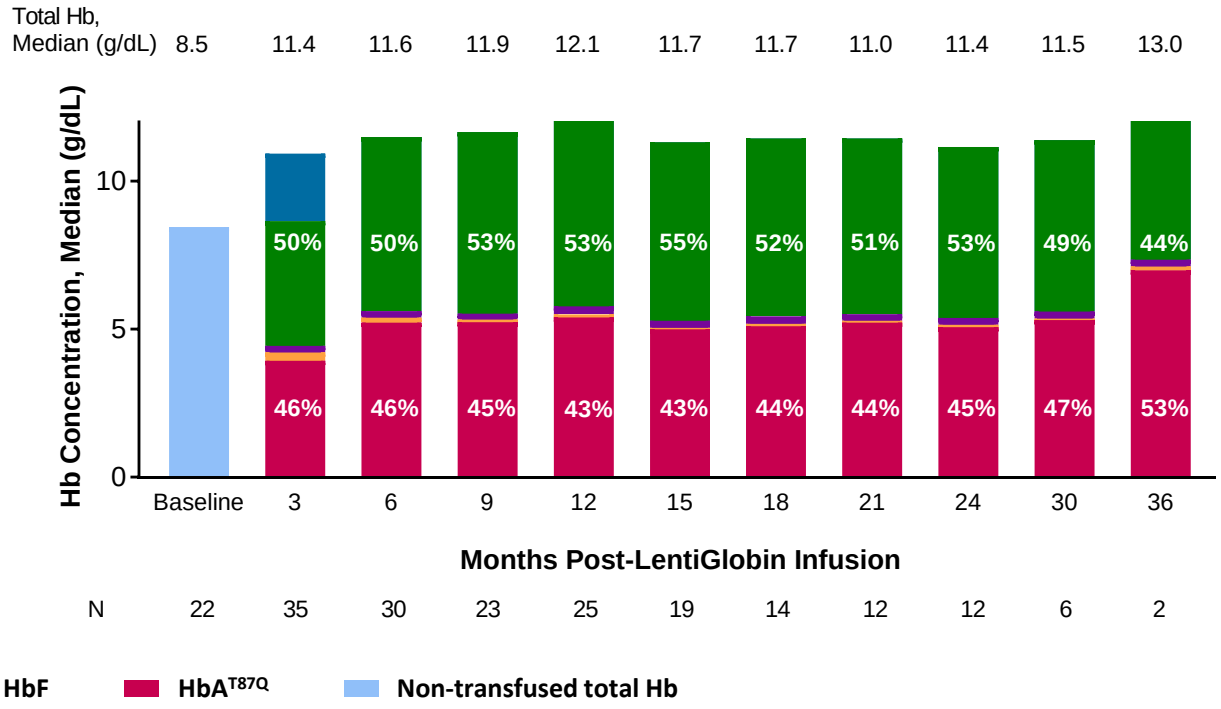
c/dg, copies per diploid genome; PB, peripheral blood; VCN, vector copy number.

HGB-206 Groups A and C: Increased HbA^{T87Q} observed in Group C post-LentiGlobin infusion

Group A*,†



Group C†

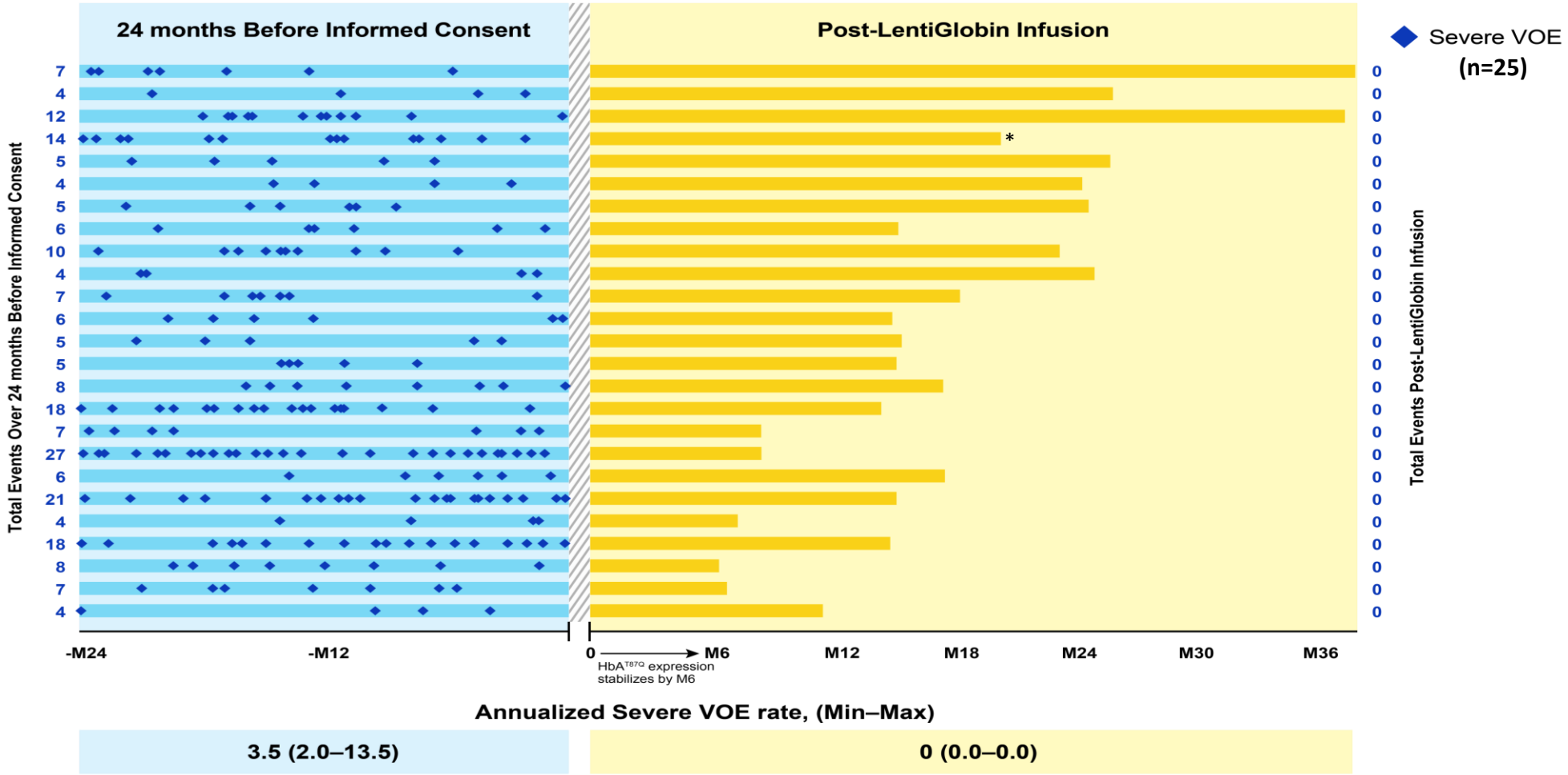


HbA (transfused) HbS HbA_2 HbF HbA^{T87Q} Non-transfused total Hb

*Two patients in Group A received transfusions post-LentiGlobin infusion for an extended period of time with an additional patient receiving transfusions at the Month 60 visit; †total Hb values include transfused Hb while bar graph values indicate non-transfused Hb at ≥6 months post-LentiGlobin infusion. Percentages represent median Hb fraction as a percentage of non-transfused total Hb; baseline is average of 2 qualified non-transfused total Hb (g/dL) in 24 months before informed consent.

g/dL, grams per deciliter; Hb, hemoglobin; HbA, adult hemoglobin; HbA^{T87Q}, Hb with modified β-globin gene ($\beta^{A\text{-}T87Q}$); HbF, fetal hemoglobin; HbS, sickle hemoglobin.

HGB-206 Group C: Complete resolution of severe VOEs ≥ 6 months post-LentiGlobin infusion



Severe VOEs were assessed in 25 patients who met the TPVOE population criterion of ≥4 severe VOEs in the 24 months before informed consent, and also met the minimum follow-up of 6 months post-LentiGlobin infusion required for VOE analysis. The hatched area represents the time between informed consent and LentiGlobin infusion, during which VOE and severe VOE data are not reported because patients received pre-harvest transfusions. A VOE was defined as an episode of acute pain with no medically determined cause other than a vaso-occlusion, including acute episodes of pain, acute chest syndrome, acute hepatic sequestration, acute splenic sequestration, and acute priapism. A severe VOE is a subset of VOEs requiring a ≥24-hour hospital or emergency room observation unit visit or ≥2 visits to a day unit or emergency room over 72 hours with both visits requiring intravenous treatment, or priapism episodes lasting >2 hours and requiring a medical facility visit. Adjudication of severe VOEs is pending.

*One death, unlikely related to LentiGlobin, 20 months post-infusion in a patient with significant baseline SCD-related cardiopulmonary disease. ACS, acute chest syndrome; HbA^{T87Q}, Hb with modified β-globin gene ($\beta^{A\text{-}T87Q}$); M, month; max, maximum; min, minimum; SCD, sickle cell disease; TP-VOE, transplant vaso-occlusive event; VOE, vaso-occlusive event.

HGB-206 Group C: Safety profile

Treatment-emergent ≥ Grade 3 AEs *Reported in ≥ 2 patients*	N=35 n (%)
Stomatitis	24 (68.6)
Thrombocytopenia	23 (65.7)
Neutropenia	19 (54.3)
Febrile neutropenia	15 (42.9)
Anemia	13 (37.1)
Leukopenia	11 (31.4)
Increased AST	6 (17.1)
Increased GGT	5 (14.3)
Nausea	4 (11.4)
Increased ALT	3 (8.6)
Decreased appetite	3 (8.6)
Abdominal pain	2 (5.7)
Increased blood bilirubin	2 (5.7)
Lymphopenia	2 (5.7)
Pharyngeal inflammation	2 (5.7)
Premature menopause	2 (5.7)
Upper abdominal pain	2 (5.7)

Serious treatment-emergent AEs *Reported in ≥ 2 patients*	N=35 n (%)
Abdominal pain	2 (5.7)
Drug withdrawal syndrome (opiate)	2 (5.7)
Nausea	2 (5.7)
Vomiting	2 (5.7)

- One patient with a nonserious Grade 2 LentiGlobin-related neutropenic fever (resolved)
- No cases of veno-occlusive liver disease
- No graft failure or vector-mediated replication-competent lentivirus
- One case of sudden death, considered unlikely related to LentiGlobin, occurred >18 months post-infusion in a patient with significant baseline SCD-related cardiopulmonary disease
- No insertional oncogenesis has been observed in Group C patients to date

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; SCD, sickle cell disease.

Data as of 17 February 2021

HGB-206 Group A: Safety profile

Treatment-emergent ≥ Grade 3 AEs *Reported in ≥ 2 patients*	**N=7** n (%)
Thrombocytopenia	6 (85.7)
Neutropenia	5 (71.5)
Stomatitis	5 (71.5)
Febrile neutropenia	4 (57.1)
Sickle cell anemia with crisis	4 (57.1)
ACS	2 (28.6)
AML	2 (28.6)
Bacteremia	2 (28.6)
Pharyngeal inflammation	2 (28.6)
Pyrexia	2 (28.6)
Serious treatment-emergent AEs *Reported in ≥ 2 patients*	**N=7** n (%)
Sickle cell anemia with crisis	4 (57.1)
ACS	2 (28.6)
AML	2 (28.6)
Bacteremia	2 (28.6)
Pyrexia	2 (28.6)

- In 2018, one patient was diagnosed with MDS ~3 years post-LentiGlobin infusion. The MDS progressed to AML and the patient died due to relapsed AML in 2020. Investigator assessed both MDS and AML as serious, Grade 4, and unlikely related to LentiGlobin for SCD
  - No vector was found in blast cells[1]
- In 2021, one patient was diagnosed with AML 5.5 years post-LentiGlobin infusion. Investigator assessed as serious, Grade 4, ongoing, and possibly related to LentiGlobin for SCD
  - AML case was unlikely related to vector[2]
  - Vector insertion in AML cells was in the *VAMP4* gene, which has no reported role in cellular proliferation or oncogenesis, and the insertion had no impact on gene expression or regulation[2]
- Both patients had classic AML driver mutations identified post-diagnosis (monosomy 7, *RUNX1*, and *PTPN11*)

ACS, acute chest syndrome; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; SCD, sickle cell disease.
1. Hsieh MM, et al. Blood Adv. 2020; 4(9): 2058–2063; 2. Tisdale J et al. Presented at ASGCT. May 11–14, 2021. Virtual

MDS/AML documented in patients following allogeneic stem cell transplantation with different conditioning regimens and with different donor sources in SCD

Donor Type Conditioning regimen	Matched 0170 TBI/ Campath	Matched 0077 TBI/Campath Pentostatin/Cy	Matched (Chicago, Riyadh) TBI/Campath	Haplo 0225 TBI/Campath -/+ Cy	Haplo 0069 TBI/Campath/Cy Pentostatin/Cy	CIBMTR (Eapen) Many types	Gene Therapies with BB305 LVV Single-agent busulfan	
							LentiGlobin for SCD‡	Beti-cel§ for TDT
N in study	58	26	64	21	19	910	**49** Median follow up (min–max) months **23.1 (0.8–74.9)**	**63** Median follow up (min–max) months **35.48 (4.1–86.5)**
MDS, AML	2 (3.5%)	1 (3.8%)	1 (1.6%)	3 (14%)*	0	6† (1.0%)	2 (4.3%)	0
	1 graft failure (MDS)	1 low engraft (AML)	1 graft failure (MDS)	2 graft failure (MDS, AML)		Details not available	2 in HGB-206 Group A[1,2]	

*Two patients with preexisting TP53 mutations; †overlap with NIH reporting in n=2; ‡as of 17 Feb 2021; §as of 21 Mar 2021.

- MDS/AML more common in patients with graft failure
- Improved engraftment in NIH haplo trial appears to have improved risk of MDS/AML
- Overall, median follow-ups not sufficient to be conclusive

AML, acute myeloid leukemia; CY, cyclophosphamide; LVV, lentiviral vector; max, maximum; MDS, myelodysplastic syndrome; min, minimum; NIH, National Institutes of Health; SCD, sickle cell disease; TBI, total body irradiation; TDT, transfusion-dependent β-thalassemia.

Data from NIH Intramural Program; 1. Hsieh MM, et al. *Blood Adv*. 2020;4:2058–63; 2. Kwiatkowski J, et al. Presented at ASPHO. April 20–23, 2021. Virtual; 3. Eapen M, et al. *Lancet Hematol*. 2019:6:e585–96; 4. Ghannam JY, et al. *Blood*. 2020:135:1185–8.

HGB-206: Potential predictors of proliferative and hematopoietic stresses post-LentiGlobin infusion

1. Transplant and associated proliferative stress
2. Continued hematopoietic stress due to minimal clinical benefit
3. Underlying increased risk of hematologic malignancies

Elevated risk of hematologic malignancies in patients with SCD following transplant, particularly suboptimal transplant[1]

Group A

1. Low CD34hi/+ cell dose
   Low transduction efficiency (DP VCN, % transduced)
2. Low PB VCN
   Low HbA^{T87Q}
   Continued VOC/ACS and hemolysis

Low median unique insertion sites

⬇

high proliferative and hematopoietic stresses

Alterations to the treatment and manufacturing process in HGB-206

Group C

1. High CD34hi/+ cell dose
   High transduction efficiency (DP VCN, % transduced)
2. High PB VCN
   High HbA^{T87Q} expression
   Complete resolution of severe VOEs
   Near normalization of hemolysis

High median unique insertion sites

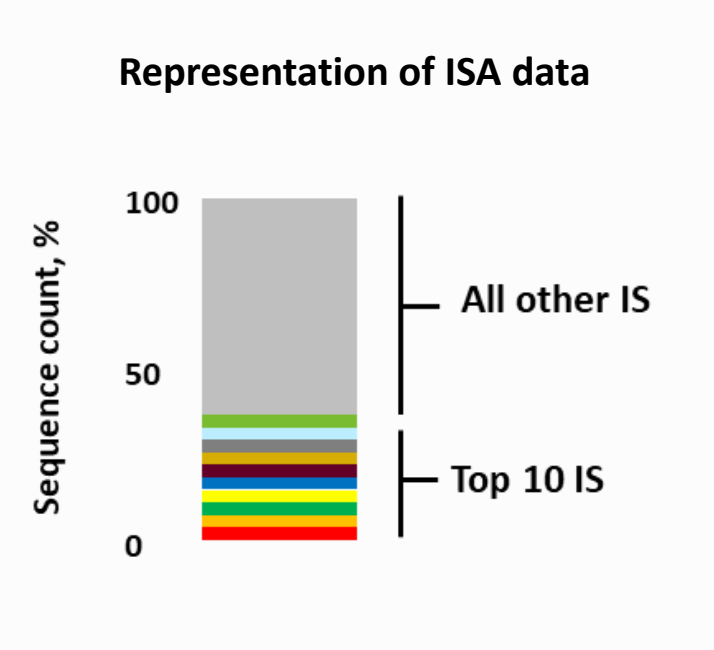
⬇

reduced proliferative and hematopoietic stresses

ACS, acute chest syndrome; HbA^{T87Q}, Hb with modified β-globin gene ($\beta^{A\text{-}T87Q}$); DP, drug product; PB, peripheral blood; SCD, sickle cell disease; VCN, vector copy number; VOC, vaso-occlusive crisis; VOE, vaso-occlusive event.

1. Tisdale JF et al. Presented at ASGCT. May 11–14, 2021. Virtual.

Utility of integration site analysis (ISA)

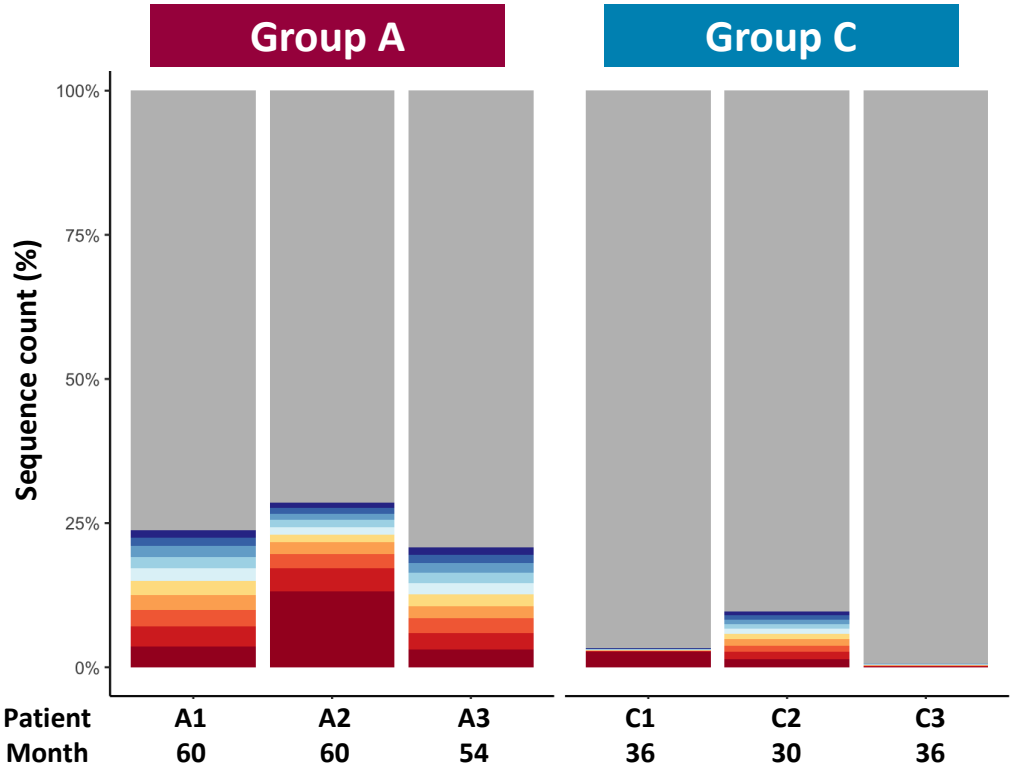


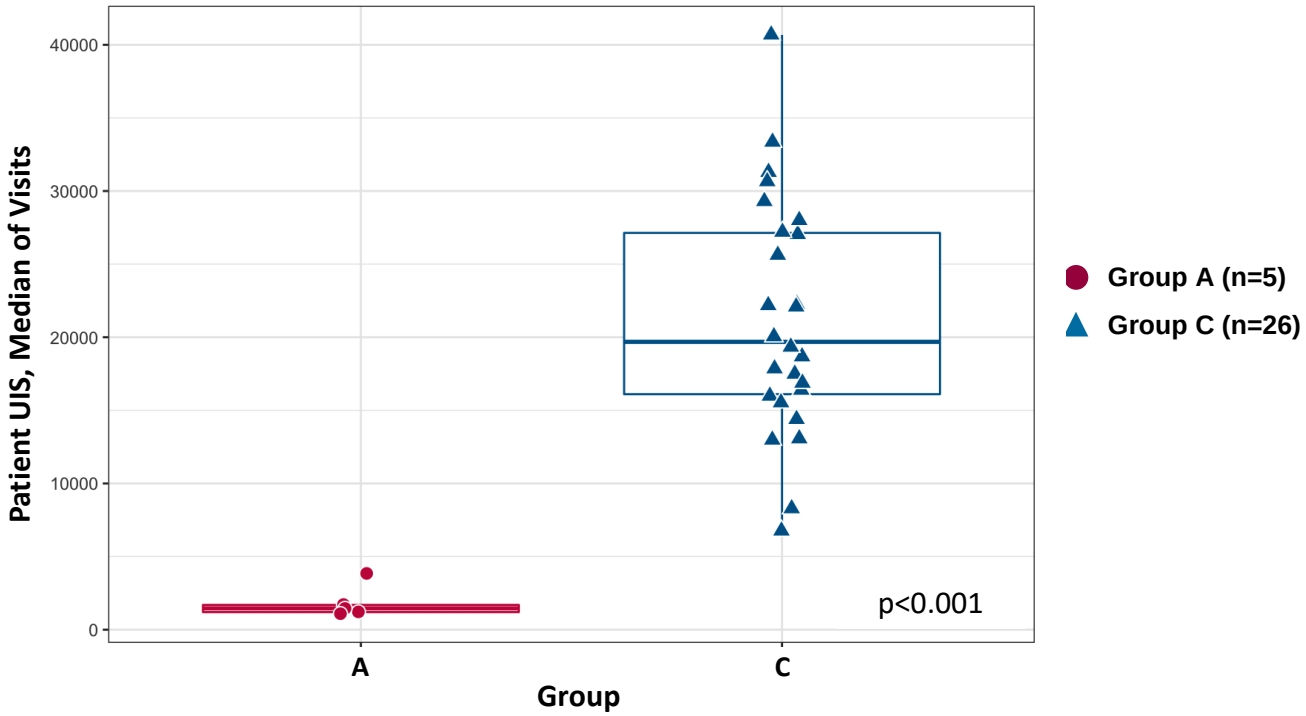
- Although the **top 10 IS** are usually the focus of integration site analyses, **all other IS** contain every other location where the transgene (ie, HbA^{T87Q}) has integrated into the genome
- This provides a **unique** fingerprint or insertional barcode of the **original** transduced CD34+ cell for **traceability**[1]
- **Progeny** containing **an IS** from the original CD34+ cell **can be tracked**, and in the event of clonal expansion or oncogenesis, this can help to determine the role, if any, of a specific IS[1,2]

HbA^{T87Q}, Hb with modified β-globin gene ($\beta^{A\text{-}T87Q}$); IS, insertion sites.

1. Biasco L et al. *Mol Ther Methods Clin Dev*. 2018; 8: 21–30. 2017; 2. Biasco L. *Hum Gene Ther*. 2017;28:1122-1129.

HGB-206 Groups A and C: Examples of ISA data



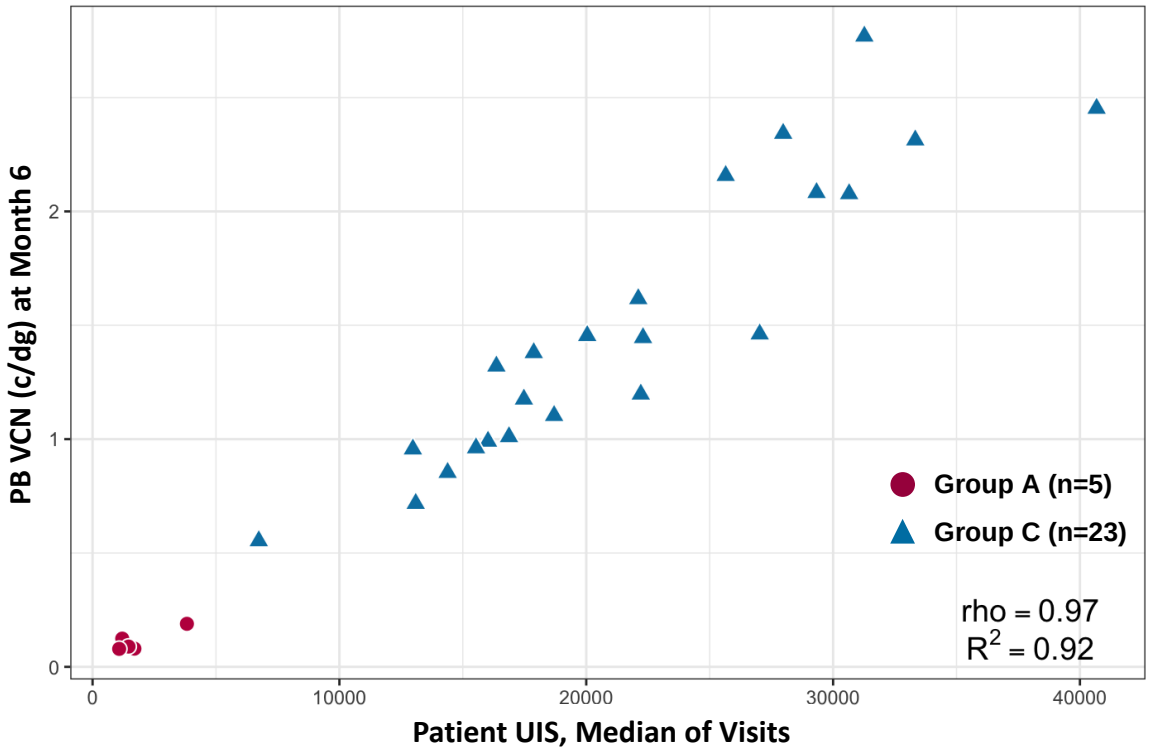


- UIS across timepoints is noisy but stable
- Top 10 IS in Group A account for higher share of detected IS
- More mappable UIS are detected in Group C
  - Increased diversity in detected UIS indicates a higher number of transduced cells engrafted in the patient
- Median UIS in Group C is 12.5-fold higher on average than in Group A. Comparison of median UIS in Group A to Group C was performed by Welch two sample t-test ($p<0.001$)

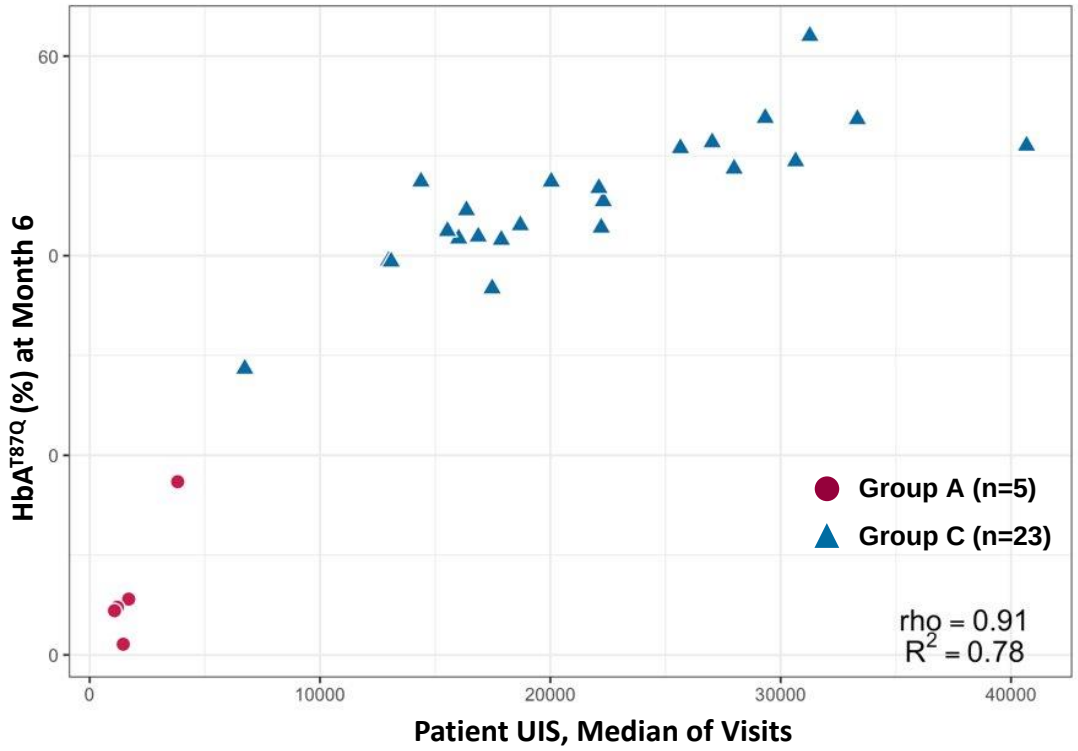
IS, insertion site; ISA, integration site analysis; UIS, unique insertion sites.

HGB-206 Groups A and C: Median unique insertion sites (UIS) correlate with PB VCN and HbA^{T87Q} at Month 6 post-LentiGlobin infusion

Median UIS vs PB VCN at Month 6



Median UIS vs HbA^{T87Q} % at Month 6

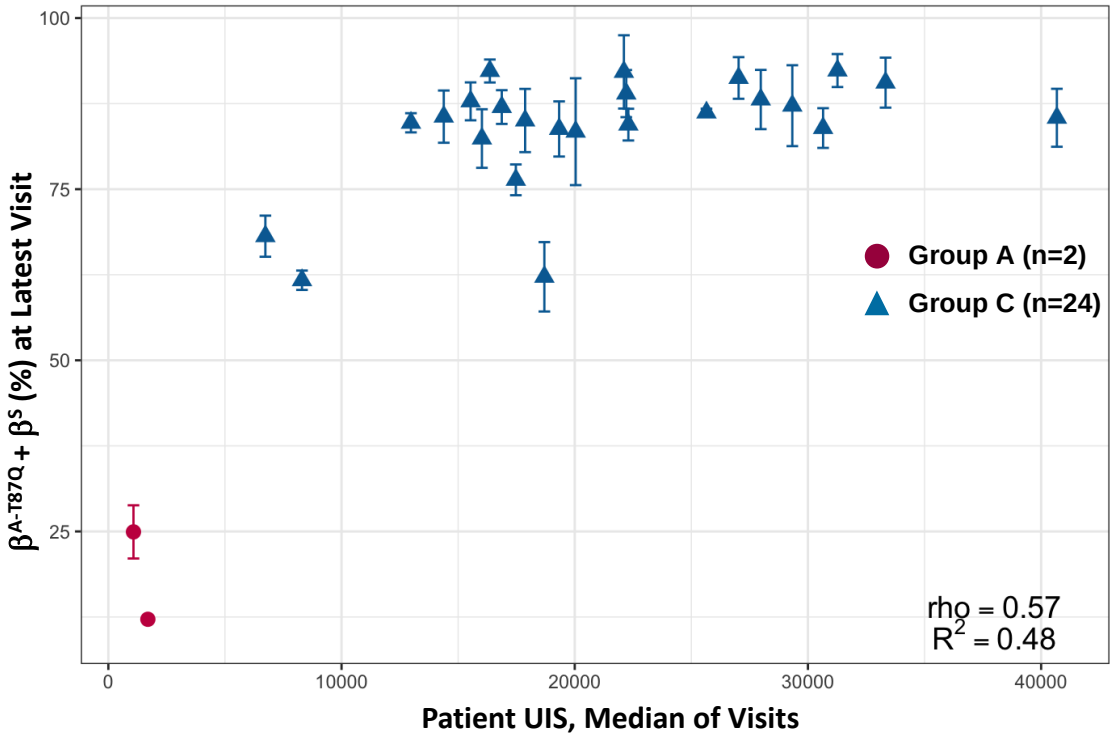


- Median UIS (as assessed by ISA with S-EPTS/LM-PCR) detected per visit for each patient and aggregated for all visits

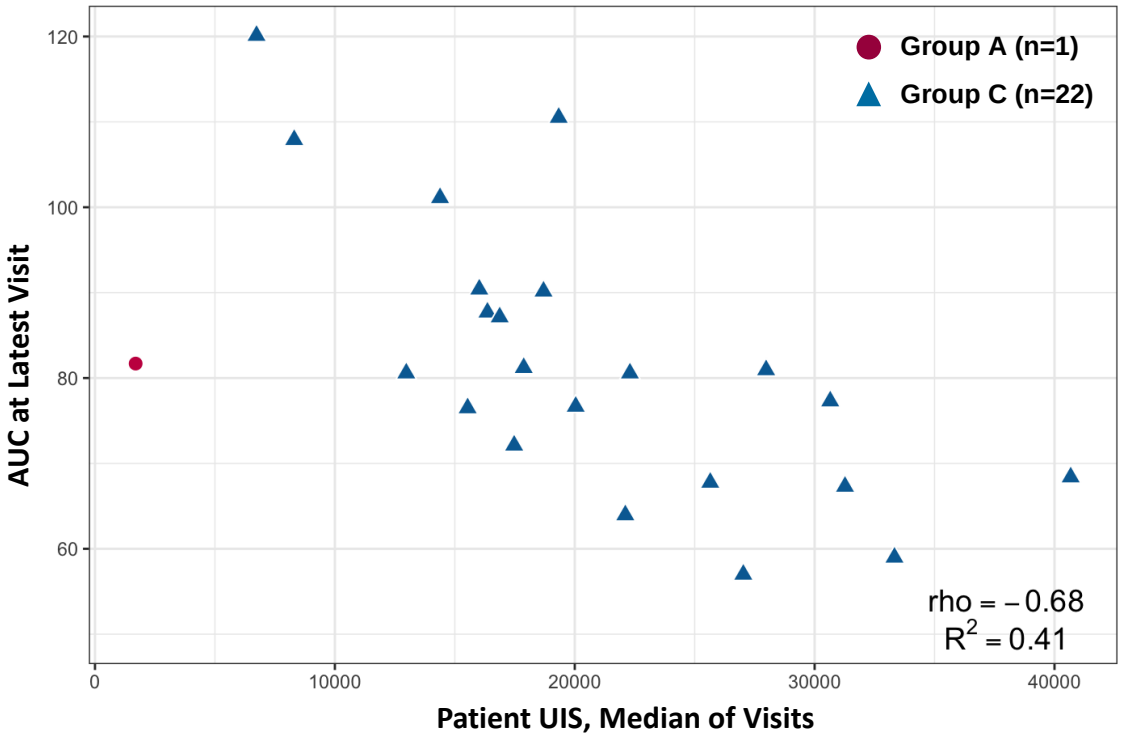
c/dg, copies per diploid genome; HbA^{T87Q}, Hb with modified β-globin gene (β^{A-T87Q}); ISA, integration site analysis; PB, peripheral blood; S-EPTS/LM-PCR, shearing extension primer tag selection ligation-mediated polymerase chain reaction; UIS, unique insertion sites; VCN, vector copy number.

HGB-206 Groups A and C: Median unique insertion sites (UIS) correlate with pancellularity and ex vivo sickling

Median UIS vs pancellularity at latest visit



Median UIS vs ex vivo sickling AUC at latest visit



- Median UIS (as assessed by ISA with S-EPTS/LM-PCR) detected per visit for each patient and aggregated for all visits
- Assays detecting presence of HbA^{T87Q} and reduced sickling effect correlate with increasing median UIS detection
- Greater diversity in detected LVV insertions are associated with increased pancellularity and reduced sickling rate

$\beta^{A\text{-}T87Q}$, modified β-globin gene; β^{S}, sickle β-globin gene ; AUC, area under the curve; HbA^{T87Q}, Hb with modified β-globin gene ($\beta^{A\text{-}T87Q}$); ISA, integration site analysis; LVV, lentiviral vector; S-EPTS/LM-PCR, shearing extension primer tag selection ligation-mediated polymerase chain reaction; UIS, unique insertion sites.

HGB-206: Summary

Group A

1. Low CD34hi/+ cell dose
   - ✓ Low clonal repertoire diversity
   - ✓ High proliferative stress

   Low transduction efficiency (DP VCN, % transduced)
   - ✓ Less polyclonal

2. Low PB VCN and low HbA^{T87Q}

   Continued VOC/ACS
   - ✓ High hematopoietic stress

   Lack of pancellular expression of HbA^{T87Q}
   - ✓ High hematopoietic stress

Low median unique insertion sites

⬇

high proliferative and hematopoietic stresses

Alterations to the treatment and manufacturing process in HGB-206

Group C

1. High CD34hi/+ cell dose
   - ✓ High clonal diversity
   - ✓ Reduced proliferative stress

   High transduction efficiency (DP VCN, % transduced)
   - ✓ Polyclonal

2. High PB VCN and HbA^{T87Q} expression

   Complete resolution of severe VOEs
   - ✓ Less hematopoietic stress

   Pancellular expression of HbA^{T87Q} and reduced sickling
   - ✓ Less hematopoietic stress

High median unique insertion sites

⬇

reduced proliferative and hematopoietic stresses

The safety profile post-LentiGlobin for all treated patients with SCD remains generally consistent with the risks of autologous stem cell transplant, myeloablative busulfan conditioning, and underlying SCD

ACS, acute chest syndrome; DP, drug product; HbA^{T87Q}, Hb with modified β-globin gene ($\beta^{A\text{-}T87Q}$); PB, peripheral blood; SCD, sickle cell disease; VCN, vector copy number; VOC, vaso-occlusive crisis; VOE, vaso-occlusive event.

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