ASH 2018 Abstract #1080

Outcomes for Initial Patient Cohorts with up to 33 Months of Follow-up in the HGB-206 Phase 1 Trial

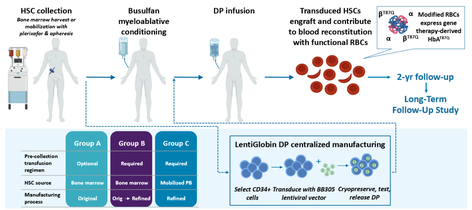
Julie Kanter,[1] John F. Tisdale,[2] Janet L. Kwiatkowski,[3,4] Lakshmanan Krishnamurti,[5] Markus Y. Mapara,[6] Manfred Schmidt,[7] Alexandra Miller,[8] Francis J. Pierciey,[8] Weiliang Shi,[8] Jean-Antoine Ribeil,[8] Mark C. Walters,[9] and Alexis A. Thompson[10, 11]

[1]Division of Pediatrics, Medical University of South Carolina, Charleston, SC; [2]Sickle Cell Branch, NHLBI/NIDDK, National Institutes of Health, Bethesda, MD; [3]Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, PA; [4]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [5]Department of Pediatrics, Division of Hematology/Oncology/BMT, Emory University School of Medicine, Atlanta, GA; [6]Columbia University College of Physicians and Surgeons, New York, NY; [7]GeneWerk GmbH, Heidelberg, Germany; [8]bluebird bio, Inc., Cambridge, MA; [9]Hematology/Oncology/BMT, UCSF Benioff Children's Hospital, Oakland, Oakland, CA; [10]Pediatric Hematology, Ann and Robert H. Lurie Children's Hospital, Chicago, IL; [11]Feinberg School of Medicine, Northwestern University, Chicago, IL

BACKGROUND

- Sickle cell disease (SCD) is caused by the polymerization of sickle hemoglobin (HbS) and expression of an anti-sickling β-globin via gene transfer into hematopoietic stem cells (HSCs) may reduce or eliminate SCD symptoms
- LentiGlobin gene therapy (GT) comprises autologous CD34+ cells transduced with the BB305 lentiviral vector, encoding human β-globin with an anti-sickling T87Q substitution (HbA^{T87Q})
- The safety and efficacy of LentiGlobin in adults with severe SCD is being evaluated in the ongoing multi-center Phase 1 study HGB-206 (NCT02140554)
- The first 7 patients (Group A) received drug product (DP) manufactured using bone marrow harvested (BMH) HSCs and demonstrated stable but inadequate HbA^{T87Q} expression
- The protocol was amended to include pre-harvest RBC transfusions, increased target busulfan levels and a refined DP manufacturing process to improve HbA^{T87Q} levels (Group B)
- The study is now enrolling patients in Group C, treated under the modified protocol and with DP manufactured from plerixafor-mobilized HSCs (these data are presented separately as abstract #1026, on Monday, December 3, Session 801, starting at 6:15 PM)
- Here, we present outcomes in the Group A and B patients with up to 3 years and > 12 months follow-up, respectively

STUDY DESIGN

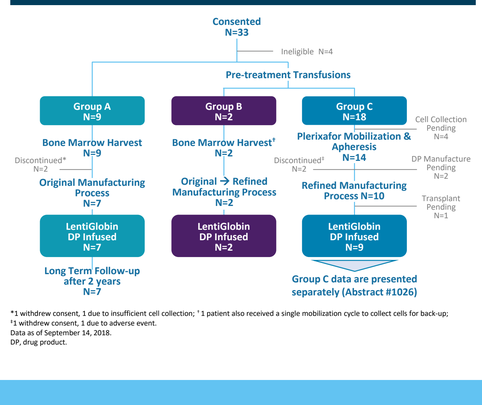


DP, drug product; HSC, hematopoietic stem cell; PB, peripheral blood; RBC, red blood cell; yr, year.

METHODS

- Phase 1 study of LentiGlobin GT in patients aged ≥ 18 years with severe SCD (history of recurrent vaso-occlusive crisis [VOC], acute chest syndrome [ACS], stroke, or tricuspid regurgitant jet velocity of > 2.5 m/s)
- For patients in Groups A and B, CD34+ HSCs were collected by BMH
- Patients received myeloablative busulfan before LentiGlobin DP was infused
- Patients in Group B (n=2 patients) were treated under modified protocol:
  - One patient (1313) received LentiGlobin GT manufactured by both the original and refined manufacturing processes
  - The other patient (1312) received LentiGlobin GT manufactured exclusively by the refined manufacturing process
- Patients were monitored for adverse events (AEs), VCN in peripheral blood (PB), HbA^{T87Q} production, and clinical and laboratory outcomes

STUDY DISPOSITION



*1 withdrew consent, 1 due to insufficient cell collection; †1 patient also received a single mobilization cycle to collect cells for back-up; ‡1 withdrew consent, 1 due to adverse event.
Data as of September 14, 2018.
DP, drug product.

RESULTS

Table 1. Patient Characteristics

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

*≥ 2 events/year in preceding 2 years; † ≥ 2 episodes in preceding 2 years, with at least one episode in the past year or in the year prior to the initiation of regular transfusions.
Data as of September 14, 2018.
ACS, acute chest syndrome; F, female; M, male; TRJV, tricuspid regurgitant jet velocity; VOC, vaso-occlusive crisis.

Table 2. Treatment Characteristics

Parameter	Group A N=7 Median (min – max)	Group B N=2 (Pt 1312, Pt 1313)
No. of bone marrow harvests	**2** (1 – 4)	**2, 3**
Busulfan AUC†, μM*min	**4747** (4084 – 5290)‡	**5256, 5017**
Follow-up, months	**29.9** (29.2 – 38.9)	**14.3, 17.2**
Neutrophil engraftment#, days	**22** (17 – 29)	**23, 28**
Platelet engraftment§, days	**56** (29 – 63)	**31, 61**
Duration of hospitalization‖, days	**37** (29 – 54)	**36, 46**

†Estimated average daily busulfan exposure over 4 days; ‡Based on 6 patients; #Absolute neutrophil count [ANC] ≥ 500 cells/μL for 3 consecutive days; §Unsupported platelet count ≥ 50,000/μL for 3 consecutive measures; ‖Initiation of hospitalization from conditioning to discharge post drug product infusion.
Data as of September 14, 2018.
AUC, area under the curve; No, number; Pt, patient.

Table 3. HGB-206 Groups A and B: Safety Associated with Bone Marrow Harvest

Grade ≥ 3 AEs	n (%) N=11
Procedural pain[1]	6 (54%)
Anemia	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.

Data as of September 14, 2018.
AE, adverse event.

- In 26 bone marrow harvests in 11 patients (9 Group A; 2 Group B), 18 grade ≥ 3 AEs were reported in 6 patients* (Table 3)
- 18 grade ≥ 3 AEs included 11 (61%) procedural pain, 3 (17%) anemia, 3 (17%) vaso-occlusive pain and 1 (6%) increase in lymphocyte count
- Plerixafor mobilization/apheresis for research purposes was performed in 1 patient, resulting in 1 additional grade 3 AE of vaso-occlusive pain

*A patient could have experienced the same AE more than once.

Table 4. HGB-206 Groups A and B: Safety Post DP Infusion

Non-hematologic grade ≥ 3 AEs *Post DP infusion reported in ≥ 2 patients*	n (%) N=9
Stomatitis	7 (78)
Febrile neutropenia	5 (56)
Vaso-occlusive pain	5 (56)
Pharyngeal inflammation	3 (33)
Bacteremia	2 (22)
Pyrexia	2 (22)

Data as of September 14, 2018.
AE, adverse event; DP, drug product.

- Grade ≥ 3 hematologic AEs were generally consistent with myeloablative busulfan conditioning and underlying SCD (Table 4)
- No graft failures or deaths reported
- 1 AE (hot flush, grade 1) was considered possibly related to DP
- No cases of vaso-occlusive liver disease (VOD) observed to date
- No vector-mediated replication-competent lentivirus (RCL) detected to date
- Continued highly polyclonal repopulation
- SAEs were reported in 8 patients, with vaso-occlusive pain (n = 5) being most common
- 1 grade 4 SAE of myelodysplastic syndrome (MDS)

A Case of Myelodysplastic Syndrome with Excess Blasts

A grade 4 SAE (serious adverse event) of MDS was reported in a Group A patient ~36 months post LentiGlobin gene therapy

- Bone marrow biopsy showed 15% myeloblasts and dysplasia
- Cytogenetics revealed monosomy 7 and abnormal chromosome 19p in 8 out of 20 metaphases

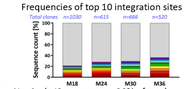
Patient and treatment characteristics

- > 40 years old at LentiGlobin infusion
- Continuous hydroxyurea (HU) for 8 years before enrollment; restarted post-LentiGlobin treatment
- Received 3.3 mg/kg (200 mg) daily intravenous busulfan conditioning over 4 days
- LentiGlobin DP characteristics: DP VCN = 1.3 copies/diploid genome; % lentiviral vector (LVV) positive cells = 29%; CD34+ cell dose = 2.8 x 106 CD34+ cells/kg

Figure 1. No Evidence of LVV-mediated Oncogenesis

No evidence of clonal dominance by insertion site (IS) analysis

Frequencies of top 10 integration sites



- No single IS represents >30% of total
- Top 5 clones consistently transitory over last 18 months of follow-up

Blast cells (CD34+) had low VCN consistent with the absence of LVV integration

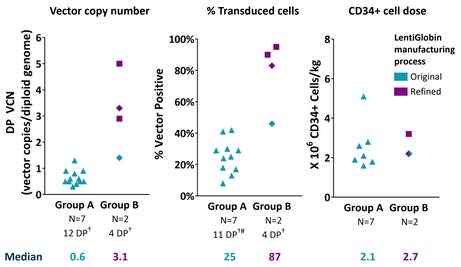
Cell populations from BM aspirate collected ~3 weeks post MDS diagnosis	Purity (%)	VCN (c/dg)
Unsorted	N/A	0.14
CD34-	98	0.21
CD34+, with myeloblasts as major contributors	93	0.02

Data as of September 14, 2018.
BM, bone marrow; c/dg, copies per diploid genome; DP, drug product; N/A, not available; VCN, vector copy number.

- Given that there is no evidence of LVV-mediated oncogenesis (Figure 1), the MDS SAE is considered unlikely related to LentiGlobin gene therapy*
- MDS has been reported in adults post autologous hematopoietic stem cell transplantation with use of alkylating agents such as busulfan[1-3]

*Per safety database.

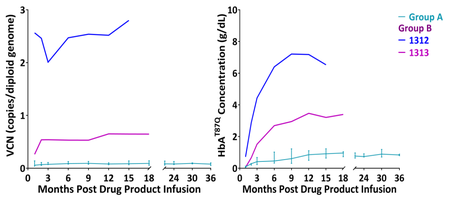
Figure 2. Refinements to Manufacturing and Cell Harvest Lead to Improved Drug Product Characteristics



Median	0.6	3.1	25	87	2.1	2.7

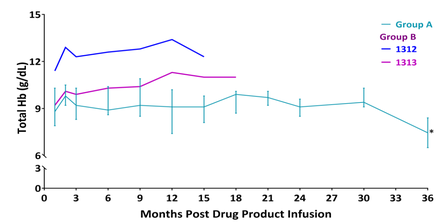
†Number of DP exceeds number of patients since some patients were harvested or mobilized more than once;
* % Transduced cells not available for 1 DP at time of analyses.
Data as of September 14, 2018.
DP, drug product.

Figure 3. Peripheral Blood VCN and HbA^{T87Q} Levels over Time



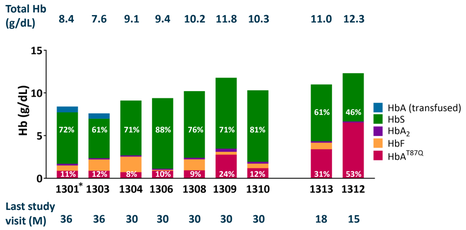
For Group A patients, medians (Q1, Q3) depicted. Group A patients with month 36 study visit (N=2).
Data as of September 14, 2018.
Hb, hemoglobin; VCN, vector copy number.

Figure 4. Total Hb Levels Are Maintained over Time



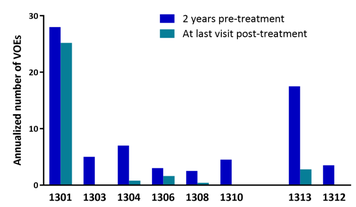
For Group A patients, medians (Q1, Q3) depicted.
†N=2 at 36 months.
Data as of September 14, 2018.
Hb, hemoglobin.

Figure 5. Vector-derived Hemoglobin in Treated Patients



*Denotes female patient.
Data as of September 14, 2018.
Hb, hemoglobin.

Figure 6. All Patients Have Decreased Rate of Annualized VOEs Post-Transplant

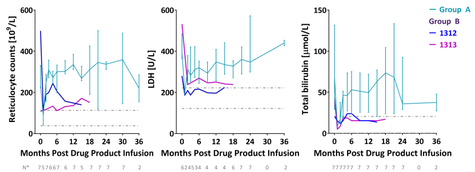


	1301	1303	1304	1306	1308	1310	1313	1312
% Decrease from baseline	10	100	89	46	84	100	84	100
Last study visit (M)	36	36	30	30	30	30	18	15

VOEs include VOCs or ACS, with VOC described as pain episode lasting ≥ 2 hours and requiring care at medical facility, and ACS defined as an acute event with pneumonia-like symptoms and the presence of a new pulmonary infiltrate.
Data as of September 14, 2018.
ACS, acute chest syndrome; RBC, red blood cell; VOCs, vaso-occlusive crises, VOEs, vaso-occlusive events.

- Patient 1309 was receiving regular RBC transfusions prior to study due to previous stroke, with no VOEs in the two years before treatment. Post-treatment, RBC transfusions were stopped and the patient had no stroke or VOEs

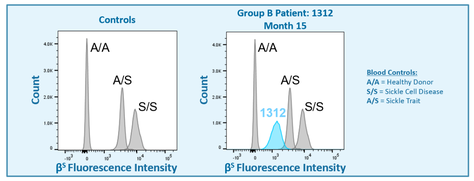
Figure 7. Hemolysis Markers over Time



Medians (Q1, Q3) depicted; Dot-dashed lines denote lower and upper limits of normal values.
*Denotes number of Group A patients with available data.
Data as of September 14, 2018.
LDH, lactate dehydrogenase.

Figure 8. Intracellular Staining of RBCs with Anti-β^S Antibody Suggests Pancellular Distribution of Gene Therapy-Derived Hb (HbA^{T87Q}) Is Achievable

- Exploratory assay: using an antibody that recognizes β^S, performed intracellular staining of RBCs followed by fluorescence-activated cell sorting (FACS) analysis
  - Fluorescence intensity (X-axis) indicates amount of β^S in cells in sample
  - Control A/A, A/S, and S/S samples showed clearly distinct β^S intensity distributions, with S/S > A/S > A/A
- Initial results in 1 Group B patient at 15 months post treatment show that nearly all RBCs have lower β^S intensity than S/S, and even A/S, samples
  - Most non-β^S globin in these samples is $\beta^{A\text{-}T87Q}$ – patients are off transfusions and HbF < 2.5% of total globin chains



Data as of September 14, 2018

SUMMARY

- Initial cohort of patients (Group A) has 30 – 36 months of follow-up:
  - Reduced VOE frequency suggests even modest HbA^{T87Q} levels (0.7 – 2.8 g/dL at last visit) have clinical effect
  - 6/7 patients have no RBC transfusions and have stable Hb over time
  - Trend towards increase in reticulocyte counts and reduction in LDH and total bilirubin concentration
- Patients in Group B, with 15 – 18 months of follow-up, appear to have improved outcomes with modified protocol and refined DP manufacturing
  - Higher HbA^{T87Q} levels ranging from 3.4 – 6.5 g/dL and higher total Hb compared to Group A
  - Hemolysis markers improved; 84 and 100% reduction in VOE frequency
- Safety profile of LentiGlobin gene therapy for severe SCD is consistent with myeloablative conditioning and underlying SCD
  - One case of MDS reported, assessed as not related to LentiGlobin gene therapy
- LentiGlobin gene therapy is being evaluated in patients in Group C, treated under a modified protocol and with DP manufactured from plerixafor-mobilized HSCs to improve outcomes (presented separately as abstract #1026, on Monday, December 3, Session 801)

REFERENCES

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

ACKNOWLEDGEMENTS

We thank all the patient participants and their families. We thank all the HGB-206 study sites and the study sponsor bluebird bio. HbS distribution experiments were performed by Liz Macari, PhD and Calvin Lee of bluebird bio. Editorial assistance for this poster was provided by Iva Kronja, PhD and Purvi Mody, PhD of bluebird bio.

DISCLOSURES

Dr. Kanter has been on the Advisory Boards for American Hematology Society, AstraZeneca, bluebird bio, National Heart, Lung, and Blood Institute, and Novartis, and has received research funding from Apopharma, bluebird bio, Global Blood Therapeutics, National Heart, Lung, and Blood Institute, Novartis, Pfizer, and Sancilio.

Presented at the 60th Annual Meeting of the American Society of Hematology (ASH) in 2018

This study is sponsored by bluebird bio