

Clinical Pharmacology BLA Review

Division of Clinical Evaluation General Medicine

Office of Clinical Evaluation

Office of Therapeutic Products

BLA	125788/0
Product	LYFGENIA (lovotibeglogene autotemcel, lovo-cel, bb1111, LentiGlobin BB305) Suspension for Intravenous Infusion, $1.7 - 20 \times 10^6$ cells/mL
Sponsor	Bluebird Bio, Inc.
Indication	Treatment of patients of 12 years of age or older with sickle cell disease and a history of vaso occlusive events (VOEs)
Date Received	April 21, 2023
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1 EXECUTIVE SUMMARY

Bluebird Bio, Inc. seeks approval of its BLA for LYFGENIA (lovotibeglogene autotemcel, lovo-cel) for treatment of patients of 12 years of age or older with sickle cell disease (SCD) and a history of vaso-occlusive events (VOEs). LYFGENIA is a gene therapy consisting of autologous CD34+ cells containing hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding β^{A-T87Q} -globin. LYFGENIA is a cell suspension for a one-time single dose intravenous infusion. The proposed minimum dose of LYFGENIA is 3.0×10^6 CD34+ cells/kg.

The clinical pharmacology evaluation of this biologics license application (BLA) is focused on data from one ongoing Phase 1/2 study (Study HGB-206, Group C) in which subjects were treated with LYFGENIA from the process proposed for commercial manufacturing – DP2a. Data from one ongoing Phase 3 study (Study HGB-210), one long-term follow-up study (Study HGB-307), one Phase 1/2 study (Study HGB-205), and a population pharmacodynamic (PD) study provided supportive evidence for approval. After infusion of LYFGENIA, transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce red blood cells (RBCs) expressing biologically active β^{AT87Q} globin. HbA^{T87Q} is then formed through the combination of 2 α -globin subunits and 2 β^{AT87Q} globin subunits. HbA^{T87Q} levels generally increase steadily after administration of LYFGENIA, and stabilize by approximately Month 6 post-infusion. In Study HGB-206 Group C,

at Month 6, the median (min, max) level of HbA^{T87Q} was 5.2 (2.6, 8.8) g/dL (N=33) and remained durable at Month 24 with median (min, max) levels of 5.5 (2.4, 9.4) g/dL (N=34). HbA^{T87Q} comprised a median (min, max) 45.7 (26.9, 63.2) (N = 34) percent of total non-transfused Hb at Month 24. Expression of HbA^{T87Q} continued to remain durable through Month 48 (N = 10), demonstrating sustained expression of the $\beta^{\text{A}^{\text{T87Q}}}$ protein derived from irreversible integration of the $\beta^{\text{A}^{\text{T87Q}}}$ globin gene into long-term hematopoietic stem cells (HSCs).

The proposed dosing regimen of LYFGENIA administered by intravenous (IV) injection has demonstrated clinical efficacy with a tolerable safety profile; therefore, the proposed dosing regimen is acceptable. From clinical pharmacology standpoint, the BLA is acceptable to support approval.

2 INTRODUCTION

Sickle cell disease (SCD) is a serious, progressive, and debilitating genetic disease caused by a point mutation in the β -globin subunit of the oxygen-carrying hemoglobin (Hb) molecule expressed in red blood cells (RBCs) and is associated with significant morbidity and early mortality. In SCD, a single point mutation within codon 6 of the β -globin gene (*HBB*) causes a glutamic acid to be replaced with valine and results in the production of an abnormal globin chain (β^{S} -globin). Patients are either homozygous for this mutation ($\beta^{\text{S}}/\beta^{\text{S}}$ or HbSS) or compound heterozygous, with one β^{S} allele and another pathogenic β -globin gene variant. High concentrations of Hb tetramers (composed of 2 β -globin and 2 α -globin subunits) that include β^{S} -globin subunits (HbS) in RBCs and subsequent polymerization under low oxygen conditions cause the RBCs to become sickled, sticky, and rigid, markedly reducing RBC lifespan, which manifests acutely as hemolytic anemia, vasculopathy, and vaso-occlusion. Repeated VOEs, progressive vasculopathy, and prolonged hemolytic anemia can result in chronic complications that lead to disease progression and end-organ damage, which are the primary causes of morbidity and mortality in adults with SCD.

LYFGENIA (lovotibeglogene autotemcel, lovo-cel, bb1111, LentiGlobin BB305) is a gene therapy designed to add functional copies of a modified β^{A} -globin gene (threonine [T] replaced with glutamine [Q] at position 87, T87Q or $\beta^{\text{A-T87Q}}$ -globin) into hematopoietic stem cells (HSCs) from patients with sickle cell disease (SCD) through transduction. LYFGENIA is an autologous CD34⁺ cells-enriched population from patients with sickle cell disease (SCD) that contains hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding $\beta^{\text{A-T87Q}}$ -globin gene, suspended in a cryopreservation solution. After LYFGENIA infusion, the transduced CD34⁺ HSCs engraft in the bone marrow and differentiate to produce red blood cells containing biologically active $\beta^{\text{A-T87Q}}$ -globin that will combine with α -globin to produce functional Hb containing $\beta^{\text{A-T87Q}}$ -globin (HbA^{T87Q}). $\beta^{\text{A-T87Q}}$ -globin can be distinguished from wildtype β^{A} -globin

and from β^S -globin through reverse-phase high-performance liquid chromatography (RPHPLC) or ultra-high performance liquid chromatography (UPLC). HbA^{T87Q} has similar oxygen-binding affinity and oxygen hemoglobin dissociation curve to wild type HbA, reduces intracellular and total hemoglobin S (HbS) levels, and is designed to sterically inhibit polymerization of HbS thereby limiting the sickling of red blood cells.

LYFGENIA is a cell suspension for a single dose (one time treatment) intravenous infusion and the proposed minimum dose of LYFGENIA is 3.0×10^6 CD34+ cells/kg.

This clinical pharmacology section of this application includes one ongoing Phase 1/2 study (Study HGB-206), with supportive data from one ongoing Phase 3 study (Study HGB-210), one long-term follow-up study (Study HGB-307), one Phase 1/2 study (Study HGB-205), and a population pharmacodynamic (PD) study.

3 SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY FINDINGS

Key clinical pharmacology findings are summarized below:

General Pharmacodynamics

- After infusion of LYFGENIA, lentiviral vector copy number levels in peripheral blood (PB VCN) increased rapidly over the first few months before reaching a plateau. At Month 6, the median (min, max) PB VCN level of DP2a product was 1.5 (0.6, 4.6) c/dg (N=38). PB VCN levels generally remained stable as of the data cutoff date for all studies, although high inter-subject variability of PB VCN kinetic profiles was observed.
- HbA^{T87Q} generally increased steadily after administration of LYFGENIA, and stabilized by approximately Month 6 post-infusion. At Month 6, the median (min, max) level of HbA^{T87Q} was 5.2 (2.6, 8.8) g/dL (N=33) and remained durable at Month 24 with median (min, max) levels of 5.5 (2.4, 9.4) g/dL (N=34) in Study HGB-206 Group C. Expression of HbA^{T87Q} continued to remain durable through Month 48 (N=10) in Study HGB-206 Group C, demonstrating sustained expression of the β^{A-T87Q} protein derived from irreversible integration of the β^{A-T87Q} -globin gene into long-term hematopoietic stem cells (HSCs).
- At Month 6 post-infusion of LYFGENIA, the median (min, max) non-transfused total Hb levels were 11.4 (5.1, 14.4) g/dL (N=33) in Study HGB-206 Group C. Non-transfused total Hb levels remained durable at Month 24 with median (min, max) levels of 11.8 (6.6, 16.2) g/dL (N=34).
- The kinetic profile of HbS was similar as HbA^{T87Q}. HbS levels increased initially after administration of LYFGENIA, and stabilized by approximately Month 6 post-infusion. At Month 6, the median (min, max) level of HbS was 5.8 (1.6, 7.3) g/dL (N=33) in Study HGB-206 Group C. HbS levels remained stable during the study. At Month 24, the median (min, max) HbS in Study HGB-206 was 5.8(1.9, 8.0) g/dL (N=34).

- The amount of each hemoglobin (Hb) fraction as well as the total Hb was generally stable by 6 months post-infusion of LYFGENIA. The relative percentages of HbA^{T87Q} and HbS were also stable over time.
- Similar results were observed for HbA^{T87Q}, non-transfused total Hb, HbS and other Hb fractions in Study HGB-210.
- Intrinsic & Extrinsic factors: various intrinsic and extrinsic factors were evaluated. Weight, race, sex, transduction efficiency (DP VCN, DP %LVV+ Cells), average busulfan area under the plasma concentration-time curve (AUC) did not impact the steady-state levels and time to steady-state for PB VCN or HbA^{T87Q}.
 - LYFGENIA manufactured from suspension culture (sLVV) and adherent culture (aLVV) had similar median values for DP VCN and DP %LVV+ Cells. Subjects who received sLVV had higher median PB VCN levels compared to subjects who received aLVV. Similar median HbA^{T87Q} levels and key efficacy endpoint (complete resolution of vaso-occlusive event, VOE-CR) were observed between sLVV and aLVV subgroups. Due to the limited sample size of subjects with sLVV (N=6), the results should be interpreted with caution.
 - Adolescent subjects had slightly higher PB VCN and HbA^{T87Q} compared to adult subjects. However, these differences were not considered to be clinically meaningful based on clinical outcomes.
 - HBA Genotype: in Study HGB-206 Group C, similar median DP VCN, DP %LVV+ Cells, PB VCN, HbA^{T87Q} values were observed for the α/α and $\alpha/-\alpha3.7$ subgroups. Two subjects with α -thalassemia trait ($-\alpha3.7/-\alpha3.7$) had higher PB VCN but lower HbA^{T87Q} levels, and lower non-transfused total Hb levels compared to α/α and $\alpha/-\alpha3.7$ groups. The rate of VOE-CR was 50%. Due to the small samples size of $-\alpha3.7/-\alpha3.7$ genotype group, no clear conclusion can be drawn.

Dosing Characteristics and Responses

- Transduction Efficiency (DP VCN and DP %LVV+ Cells) and PB VCN: Both drug product vector copy number (DP VCN) and the percentage of transduced cells in drug product (DP %LVV+Cells) measure drug product characteristics related to transduction efficiency. Positive non-linear correlative relationship was observed between DP VCN and DP %LVV+ cells: DP %LVV+ cells increased rapidly with the increase of DP VCN at low DP VCN levels, and then trended to plateau at higher levels of DP VCN. Both DP VCN and DP %LVV+ Cells positively correlated (linear relationship) with PB VCN at Month 6 after LYFGENIA infusion in DP2a pool, with DP %LVV+Cells showing the stronger correlation.
- Transduction Efficiency (DP VCN and DP %LVV+Cells) and %HbA^{T87Q}: DP %LVV+ Cells, but not DP VCN showed a significant correlation (linear relationship) with %HbA^{T87Q} in non-transfused Hb at Month 6 after LYFGENIA infusion in DP2a pool: subjects who received LYFGENIA with higher DP %LVV+Cells had higher HbA^{T87Q} at Month 6, compared to subjects who received LYFGENIA with lower DP %LVV+Cells.
- Total cell dose (CD34+ cells/kg) and %HbA^{T87Q}: there was no correlation between total cell dose (CD34+ cells/kg) and %HbA^{T87Q} in peripheral blood at Month 6 in DP2a pool subjects,

suggesting that the lowest cell dose evaluated to date was adequate for effective reconstitution of HSCs in treated subjects.

- Busulfan exposure (AUC) and Day of engraftment: there was no correlation observed between the estimated average busulfan exposure (area under the curve, AUC) and the Day of engraftment (neutrophil and platelet) for the SCD and DP2a pools. All subject received LYFGENIA had successful engraftment. The targeted AUC range of busulfan evaluated in clinical studies was considered adequate for myeloablation.
- Total cell dose (CD34+ cells/kg) and Day of engraftment: there was no correlation observed between total cell dose (CD34+ cells/kg) and the Day of engraftment (neutrophil and platelet) for the SCD and DP2a pools, indicating even the lowest cell doses evaluated were adequate for effective reconstitution of HSCs in treated subjects.
- Dosing characteristics (DP VCN, DP %LVV+Cells and total cell dose) and complete resolution of vaso-occlusive events (VOE-CR): there was no significant correlation observed between LYFGENIA dosing characteristics and complete resolution of vaso-occlusive events (VOE-CR).

Pharmacodynamic Responses (at Month 6) and Complete Resolution of Vaso-occlusive Event (VOE-CR)

- PB VCN and %HbA^{T87Q}: %HbA^{T87Q} increased quickly with the increase of PB VCN at lower PB VCN levels, followed by a %HbA^{T87Q} plateau at higher PB VCN levels in all SCD pool. Similar trend was observed in DP2a pool. This observation reflected the feedback regulation of β -globin levels within erythroid cells. Population PD modeling also showed similar results that steady-state PB VCN was an influential covariate for HbA^{T87Q}.
- PB %LVV+ Cells and %HbA^{T87Q}: %HbA^{T87Q} increased linearly with the increase of PB %LVV+ Cells. The significant relationship was observed in both SCD pool and DP2a pool.
- Non-transfused total Hb and complete resolution of vaso-occlusive event (VOE-CR): in the evaluable subjects, 4 of 14 (29%) subjects with lower than median level of non-transduced total Hb (11.4 g/dL) did not achieve VOE-CR. All 15 subjects with higher than and median levels of non-transduced total Hb achieved VOE-CR.
- HbA^{T87Q} at Month 6 and resolution of vaso-occlusive event (VOE-CR): in the evaluable subjects, 3 of 14 (21%) subjects with lower than median level of HbA^{T87Q} (5.2 g/dL) did not achieve VOE-CR. One of 15 (7%) subjects with higher than and median levels of HbA^{T87Q} did not achieve VOE-CR.

4 LABELING COMMENTS

The clinical pharmacology reviewer has reviewed the package insert for BLA 125788/0 and finds it acceptable with incorporating following comments.

12. CLINICAL PHARMACOLOGY

12.1. Mechanism of Action

Recommended to delete the promotional language.

12.2. Pharmacodynamics

Recommended to update the pharmacodynamic information using data with cutoff date of February 2023.

5 RECOMMENDATIONS

The clinical pharmacology information in this BLA is acceptable. Please refer to section 4 for detailed Labeling Recommendations.

6 COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

6.1 Overview of Clinical Pharmacology Evaluation

The clinical pharmacology review team's recommendation for approval of LYFGENIA is based on review of data from the one ongoing Phase 1/2 study (Study HGB-206), with supportive data from one ongoing Phase 3 study (Study HGB-210), one long-term follow-up study (Study HGB-307), one Phase 1/2 study (Study HGB-205) (Table 1). The Applicant also developed a population pharmacodynamic (PD) model to characterize LYFGENIA PD in subjects with SCD. LYFGENIA from different manufacturing processes were used in the clinical development. Clinical pharmacology evaluation (except for dosing characteristics- and pharmacodynamic markers-related correlative analysis) focuses on LYFGENIA from the process proposed for commercial manufacturing – DP2a (Study HGB-206 Group C).

Study Design

As shown in Figure 1, all parent clinical studies had a similar study design with 4 main stages:

1. screening to determine eligibility,
2. apheresis after mobilization with granulocyte colony-stimulating factor (G-CSF) and plerixafor to collect cells for drug product manufacture,
3. myeloablation of the subjects using busulfan to deplete endogenous HSCs (thus allowing repopulation of the subject with HSCs containing the transgene), followed by IV infusion of manufactured drug product as a single dose on Day 1. Busulfan concentrations were monitored and busulfan dosing was adjusted to target recommended AUC to effectively remove endogenous untransduced HSCs.
4. follow-up of approximately 24 months in the parent study.

Table 2 summarizes demographics of the clinical studies.

Table 1. Clinical studies conducted to characterize clinical pharmacology of LYFGENIA in Subjects with SCD

Study Identifier (Status) Location of CSR	Study Title	Number of Subjects and Age Range^a	Drug Product Characteristics and Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) for Subjects with SCD from Study Protocol or Study Objective	Interim Data Cut-off Date for Ongoing Studies
HGB-205 (completed: 26 February 2019) 5.3.5.2 CSR HGB-205	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β -Hemoglobinopathies (Sickle Cell Anemia and β -Thalassemia Major) by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral β^{A-T87Q} -globin Globin Vector (LentiGlobin BB305 Drug Product)	7 planned (SCD or TDT ^b) 3 subjects with SCD were treated and completed the study (aged 13, 16, and 21 years)	Manufacturing Process 0 Autologous cell source: bone marrow harvest Cell dose: $\geq 2.0 \times 10^6$ CD34+ cells/kg	4000 to 5200 $\mu\text{M} \cdot \text{min}^c$	<i>For all subjects^d:</i> RBC transfusion requirements (measured in mL/kg) per month and per year post-transplant. Number of total in-patient hospitalization days (post- transplant discharge) at 6, 12, and 24 months. <i>For severe SCD subjects:</i> Number of VOC or ACS events at 6, 12, and 24 months. Evaluation of changes in the nature or frequency of the subject- specific main inclusion criteria.	NA; study complete

Study Identifier (Status) Location of CSR	Study Title	Number of Subjects and Age Range ^a	Drug Product Characteristics and Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) for Subjects with SCD from Study Protocol or Study Objective	Interim Data Cut-off Date for Ongoing Studies
HGB-206 (study ongoing; enrollment and treatment completed) 5.3.5.2 Interim CSR HGB-206	A Phase 1/2 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the LentiGlobin BB305 Lentiviral Vector in Subjects with Severe Sickle Cell Disease	Approximately 50 subjects ≥ 12 and ≤ 50 years of age with SCD planned 45 subjects treated (7 in Group A; 2 in Group B; 36 in Group C) 38 subjects completed	Manufacturing Process 1 (Group A and B), 2 (Group B), and 2a (Group C) Autologous cell source: bone marrow harvest for Groups A and B; plerixafor-mobilized cells for Group C Cell dose: $\geq 1.5 \times 10^6$ CD34+ cells/kg (Group A); $\geq 2.0 \times 10^6$ CD34+ cells/kg (Group B); $\geq 3.0 \times 10^6$ CD34+ cells/kg (Group C)	4400 to 5400 $\mu\text{M} \cdot \text{min}^c$	Endpoint: VOE-CR, defined as complete resolution of VOEs, between 6 months and 18 months after drug product infusion	11 August 2022
HGB-210 (ongoing) 5.3.5.2 Interim CSR HGB-210	A Phase 3 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the BB305 Lentiviral Vector in Subjects with Sickle Cell Disease	Approximately 35 adults and pediatric subjects ≥ 2 and ≤ 50 years of age with SCD planned 2 subjects treated 0 subjects completed	Manufacturing Process 2a Autologous cell source: plerixafor-mobilized cells $\geq 3.0 \times 10^6$ CD34+ cells/kg	4400 to 5400 $\mu\text{M} \cdot \text{min}$	Endpoint: The proportion of subjects achieving VOE-CR, defined as complete resolution of VOEs, between 6 months and 18 months after drug product infusion.	01 August 2022

Study Identifier (Status) Location of CSR	Study Title	Number of Subjects and Age Range ^a	Drug Product Characteristics and Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) for Subjects with SCD from Study Protocol or Study Objective	Interim Data Cut-off Date for Ongoing Studies
LTF-307 ^e (ongoing) 5.3.5.2 Interim CSR LTF-307	Long-term Follow-up of Subjects With Sickle Cell Disease Treated With Ex Vivo Gene Therapy Using Autologous Hematopoietic Stem Cells Transduced With a Lentiviral Vector	Dependent on the number of subjects completing (or discontinuing) each parent study. 41 subjects enrolled 0 subjects completed	No investigational treatment is administered in this study.	NA	Objective: Evaluate long-term efficacy of the drug product No primary efficacy endpoint Note: CSR included the same VOE-CR endpoint as detailed for the Phase 1/2 Study HGB-206 and Phase 3 Study HGB-210	18 August 2022

Abbrev.: ACS, acute chest syndrome; AUC, area under the curve; CD, cluster of differentiation; CSR, clinical study report; NA, not applicable; RBC, red blood cells; SCD, sickle cell disease, TDT, transfusion-dependent β -thalassemia, VOC, vaso-occlusive crisis; VOE, vaso-occlusive event; VOE-CR, complete resolution of vaso-occlusive events

^a As of the interim or final data cut-off date for each study as noted above.

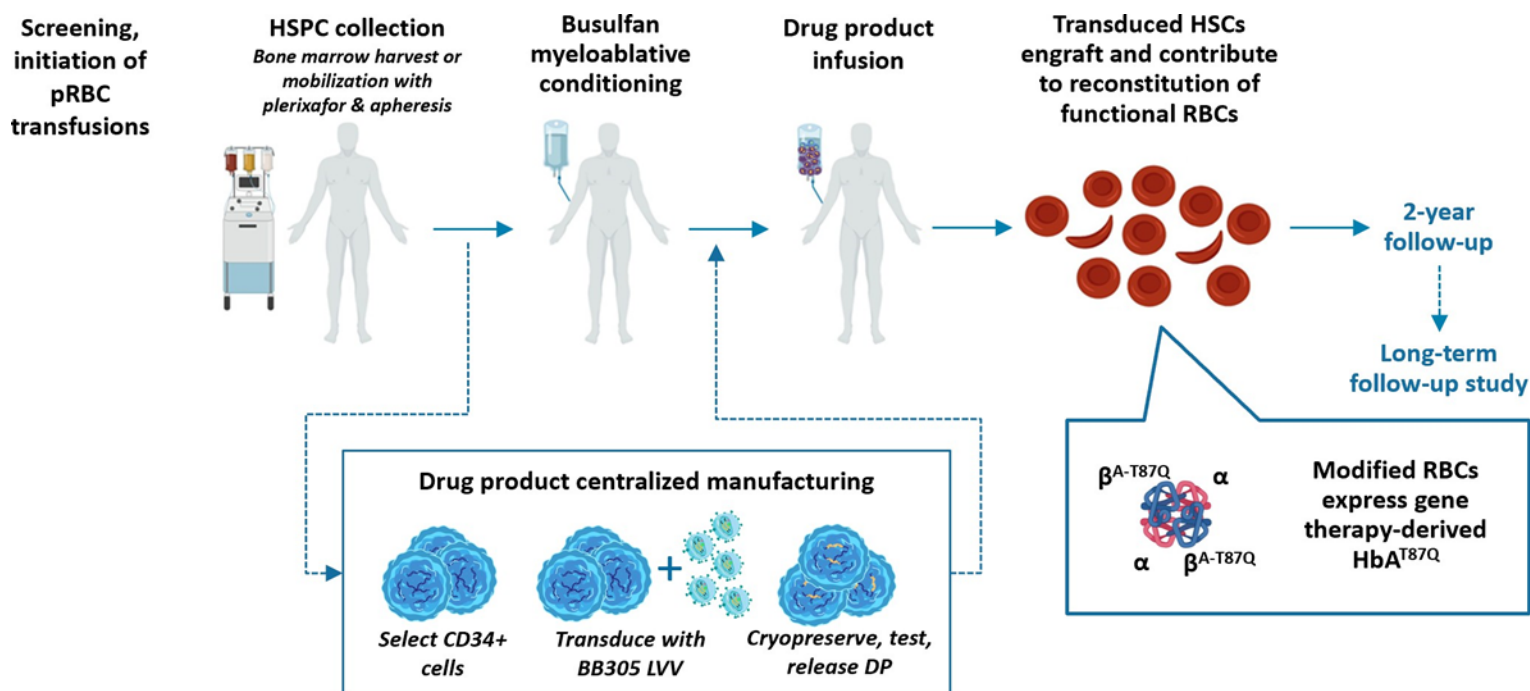
^b Subjects with TDT (N = 4 treated in Study HGB-205) were not included in the pharmacodynamic analysis for this module.

^c Changed from 3200 to 4400 $\mu\text{M} \cdot \text{min}$ to 4000 to 5200 $\mu\text{M} \cdot \text{min}$ in Protocol HGB-205 Version 7.0 (19 May 2016). Changed from 3600 to 5000 $\mu\text{M} \cdot \text{min}$ to 4000 to 5000 $\mu\text{M} \cdot \text{min}$ in Protocol HGB-206 Version 5.0 (04 September 2015) and from 4000 to 5000 $\mu\text{M} \cdot \text{min}$ to 4400 to 5400 $\mu\text{M} \cdot \text{min}$ in Protocol HGB-206 Version 6.0 (23 June 2016).

^d Protocol HGB-205 did not have a primary endpoint but had 4 parallel efficacy endpoints for subjects with SCD.

^e Twenty-one subjects were initially enrolled in Study LTF-303, a long-term follow-up study for subjects who received treatment for either TDT or SCD. All subjects treated with lovo-cel for SCD were subsequently enrolled in Study LTF-307, as applicable, and their data were transferred to the LTF-307 database

Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

Figure 1. Schematic of Study Design

Abbrev.: CD, cluster of differentiation; DP, drug product; Hb, hemoglobin; HbA^{T87Q}, hemoglobin A that contains β^{A-T87Q} -globin; HbS, hemoglobin S; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; LVV, lentiviral vector; pRBC, packed red blood cells; RBC, red blood cells Notes: Periods estimated for each Study Stage are approximate. Stem cell collection in Stage 2 used bone marrow harvest for Studies HGB-205 and HGB-206 Groups A and B, whereas Studies HGB-206 Group C and HGB-210 used apheresis to collect stem cells mobilized using plerixafor. Stage 2 may need to be repeated to obtain sufficient cells. Subjects underwent a schedule of pRBC transfusions starting at least 60 days prior to stem cell collection and continuing through until the start of conditioning, to reach a target Hb of 10 g/dL prior to mobilization and 8 to 10 g/dL prior to conditioning, with specified pre-transfusion HbS targets.

Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

Table 2. Demography of Clinical Study Subjects

Parameter	Study						Manufacturing Process		Overall
	HGB-205 (N = 3)	HGB-206 Group A (N = 7)	HGB-206 Group B (N = 2)	HGB-206 Group C (N = 36)	HGB-206 Overall (N = 45)	HGB-210 (N = 2)	DP0/1/2 (N = 12)	DP2a (N = 38)	SCD (N = 50)
Age at Informed Consent or Assent (years)^a									
n	3	7	2	36	45	2	12	38	50
Median	16.0	26.0	24.5	24.0	25.0	16.0	23.5	23.5	23.5
Min, Max	13, 21	18, 42	22, 27	12, 38	12, 42	15, 17	13, 42	12, 38	12, 42
Age at Informed Consent or Assent (Category), n (%)^a									
≥ 12 years	3 (100.0)	7 (100.0)	2 (100.0)	36 (100.0)	45 (100.0)	2 (100.0)	12 (100.0)	38 (100.0)	50 (100.0)
≥ 18 years	1 (33.3)	7 (100.0)	2 (100.0)	28 (77.8)	37 (82.2)	0	10 (83.3)	28 (73.7)	38 (76.0)
≥ 12 years to < 18	2 (66.7)	0	0	8 (22.2)	8 (17.8)	2 (100.0)	2 (16.7)	10 (26.3)	12 (24.0)
Sex, n (%)									
Male	1 (33.3)	6 (85.7)	2 (100.0)	22 (61.1)	30 (66.7)	1 (50.0)	9 (75.0)	23 (60.5)	32 (64.0)
Female	2 (66.7)	1 (14.3)	0	14 (38.9)	15 (33.3)	1 (50.0)	3 (25.0)	15 (39.5)	18 (36.0)
Race, n(%)									
Asian	0	0	1 (50.0)	0	1 (2.2)	0	1 (8.3)	0	1 (2.0)
Black or African American	2 (66.7)	7 (100.0)	1 (50.0)	35 (97.2)	43 (95.6)	2 (100.0)	10 (83.3)	37 (97.4)	47 (94.0)
White	1 (33.3)	0	0	0	0	0	1 (8.3)	0	1 (2.0)
Not Reported	0	0	0	1 (2.8)	1 (2.2)	0	0	1 (2.6)	1 (2.0)
Ethnicity, n (%)									
Hispanic	0	0	0	1 (2.8)	1 (2.2)	0	0	1 (2.6)	1 (2.0)
Not Hispanic	3 (100.0)	7 (100.0)	2 (100.0)	33 (91.7)	42 (93.3)	2 (100.0)	12 (100.0)	35 (92.1)	47 (94.0)
Not Reported	0	0	0	2 (5.6)	2 (4.4)	0	0	2 (5.3)	2 (4.0)

Parameter	Study						Manufacturing Process		Overall
	HGB-205 (N = 3)	HGB-206 Group A (N = 7)	HGB-206 Group B (N = 2)	HGB-206 Group C (N = 36)	HGB-206 Overall (N = 45)	HGB-210 (N = 2)	DP0/1/2 (N = 12)	DP2a (N = 38)	SCD (N = 50)
Genotype for β-Globin									
β^S/β^S	2 (66.7)	7 (100.0)	2 (100.0)	36 (100.0)	45 (100.0)	2 (100.0)	11 (91.7)	38 (100.0)	49 (98.0)
β^S/β^0	1 (33.3)	0	0	0	0	0	1 (8.3)	0	1 (2.0)
Genotype for α-Globin									
$\alpha\alpha/\alpha\alpha$	2 (66.7)	6 (85.7)	1 (50.0)	23 (63.9)	30 (66.7)	1 (50.0)	9 (75.0)	24 (63.2)	33 (66.0)
$\alpha\alpha/-\alpha3.7$	1 (33.3)	1 (14.3)	1 (50.0)	11 (30.6)	13 (28.9)	1 (50.0)	3 (25.0)	12 (31.6)	15 (30.0)
$-\alpha3.7/-\alpha3.7$	0	0	0	2 (5.6)	2 (4.4)	0	0	2 (5.3)	2 (4.0)

Abbrev.: DP0/1/2, drug manufacturing process 0, 1 or 2; DP2a, drug manufacturing process 2a; max, maximum; min, minimum; SCD, sickle cell disease;

TP, transplant population

Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

6.2 Dosing Characteristics

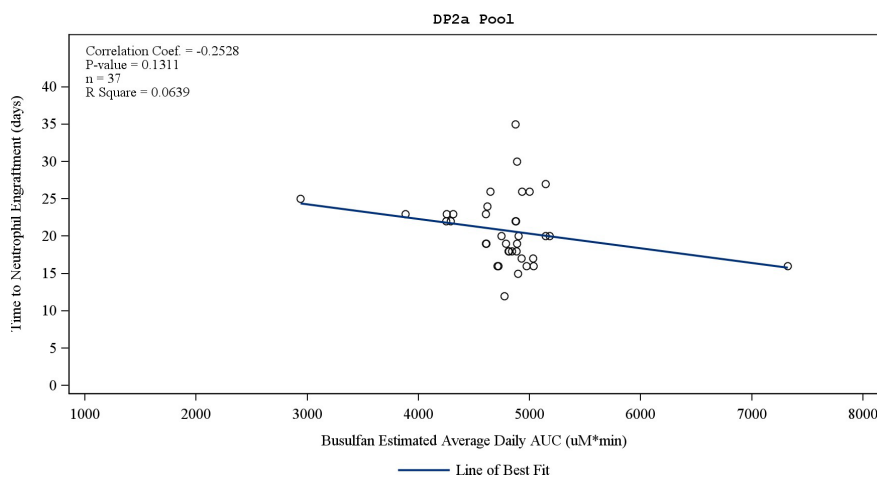
6.2.1 Busulfan

Prior to infusion of LYFGENIA, busulfan was administered intravenously (IV) to study subjects for myeloablation to deplete endogenous HSCs, to allow repopulation of the subject with HSCs containing the transgene without dilution due to the presence of unablated cells. Busulfan was administered at a starting dose of 3.2 mg/kg/day via a single daily intravenous (IV) infusion, or at 0.8 mg/kg every 6 hours for 4 consecutive days (Day -6 through Day -3). The dose of busulfan was adjusted based on PK monitoring in order to maintain appropriate levels for myeloablation (area under the curve [AUC] goal of 1250 [range 1110 to 1350] $\mu\text{M}\cdot\text{min}$ for a q6h dosing regimen, or 5000 [range 4400 – 5400] $\mu\text{M}\cdot\text{min}$ for a qd dosing regimen). The dosage was calculated based on ideal versus actual body weight.

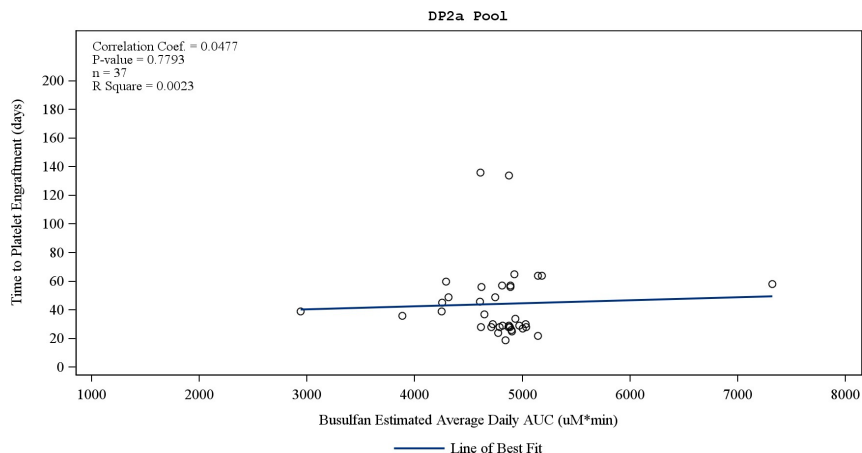
The relationship between conditioning busulfan AUC and engraftment was evaluated. No correlation was observed between the estimated average daily busulfan AUC and the Day of either neutrophil or platelet engraftment for the SCD and DP2a Pools (Figure 2). These results indicate that busulfan AUC did not affect the kinetics of either neutrophil or platelet engraftment within the AUC range observed during these studies. This may be due to the fact that the AUC range of busulfan used in the clinical studies was narrow.

Figure 2. Busulfan AUC versus Day of Engraftment (DP2a Pool)

a. Day of Neutrophil Engraftment



b. Day of Platelet Engraftment



Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

6.2.2 LYFGENIA (lovo-cel)

The minimum dose of CD34+ cells to be administered per subject is based on accepted safe practice to achieve rapid and robust hematopoietic reconstitution with long-term engraftment after autologous transplantation. There is a consensus in the field that the minimum CD34+ dose of mobilized peripheral blood stem cells associated with favorable engraftment kinetics is $\geq 1.5 \times 10^6$ cells/kg; lower cell doses can result in engraftment, but there are delays in neutrophil and platelet recovery relative to higher doses.

The manufacturing process of LYFGENIA was optimized during the drug development. LYFGENIA from Process 0 (DP0) was used in the proof-of-concept Study HGB-205. Process 1 (DP1) was used to manufacture product for Study HGB-206 Group A and one lot in Group B. LYFGENIA from Process 2 (DP2) was administered in subjects of Study HGB-206 Group B. The commercial manufacturing process (DP2a) was used to produce LYFGENIA in Study HGB-206 Group C and Study HGB-210.

During early development, bone marrow harvest was used as the source of autologous cells for drug product manufacture (HGB-205, HGB-206 Groups A and B). The cell dose was $\geq 1.5 \times 10^6$ CD34+ cells/kg for Study HGB-206 Group A and $\geq 2.0 \times 10^6$ CD34+ cells/kg for Study HGB-205 and Study HGB-206 Group B. Subsequently, the source of autologous cells was changed to plerixaflor mobilized cells collected by apheresis. Due to higher CD34+ cell yields with this cell source, the cell dose was increased to $\geq 3.0 \times 10^6$ CD34+ cells/kg (Study HGB-206 Group C and Study HGB-210). The lowest dose received by any subject that used plerixaflor- mobilized cells as an autologous cell source was 3.0×10^6 CD34+ cells/kg. As shown in Table 3, the median (min, max) total cell dose of LYFGENIA (lovo-cel) (DP2a manufacturing process) administered to subjects with SCD was 6.1 (3.0, 14.0) $\times 10^6$ CD34+ cells/kg (N=38). Compared to DP0/1/2

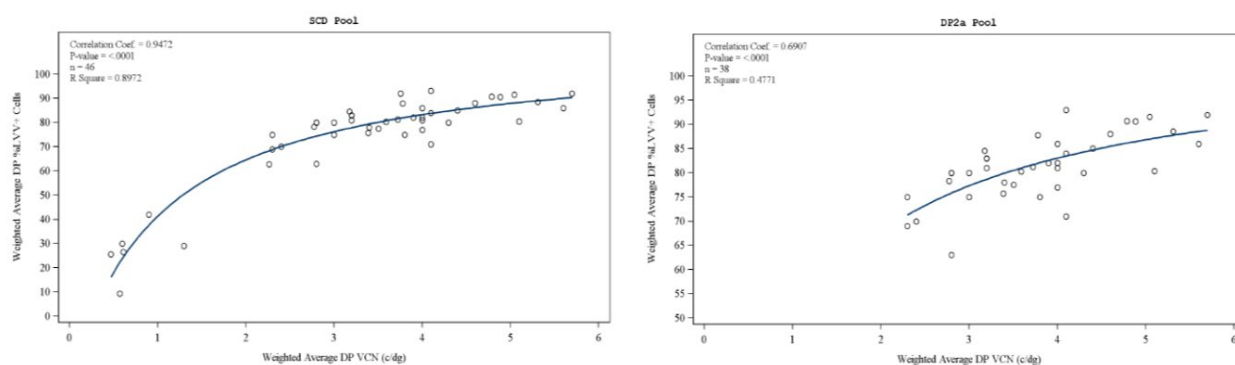
process products, LYFGENIA from DP2a process has higher DPVCN, DP %LVV+ cells and DP VCN/DP %LVV+ cells (Table 3).

During Study HGB-206, LVV manufacturing transitioned from using adherent cell cultures (aLVV) to using cells in suspension (sLVV) to allow scale-up for commercial manufacturing. Six subjects received LYFGENIA with sLVV. DP VCN values of these 6 subjects (median [min, max]: 4.45 [3.0, 5.3] c/dg) were within the range of values obtained for all subjects using Process 2a (median [min, max]: 3.79[2.3, 5.7] c/dg).

DP VCN versus DP %LVV+ Cells

DP VCN and DP %LVV+ Cells both measure characteristics of the drug product that are related to transduction efficiency. As depicted in Figure 3, DP %LVV+ Cells increases sharply with DP VCN at low DP VCN and then trends to plateau at higher levels of DP VCN. This non-linear relationship suggests that increases in DP VCN reflect increases in the number of copies per cell as well as increases in the percentage of transduced cells and that, as the percentage of transduced cells approaches 100%, a rise in DP VCN increasingly reflects an increase in the number of copies per cell.

Figure 3. DP VCN versus DP %LVV+ Cells



Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

The relationships between LYFGENIA dosing characteristics and PD responses are analyzed and discussed in Section 6.5.

Table 3. Dosing Characteristics of LYFGENIA

Parameter	Study						Manufacturing Process		Overall
	HGB-205 (N = 3)	HGB-206 Group A (N = 7)	HGB-206 Group B (N = 2)	HGB-206 Group C (N = 36)	HGB-206 Overall (N = 45)	HGB-210 (N = 2)	DP0/1/2 (N = 12)	DP2a (N = 38)	SCD (N = 50)
Total Dose (CD34+ cells × 10⁶/kg)^a									
n	3	7	2	34	43	2	12	36	48
Median	4.74	2.10	2.70	6.40	5.20	4.25	2.70	6.10	5.15
Min, Max	3.0, 5.6	1.6, 5.1	2.2, 3.2	3.0, 14.0	1.6, 14.0	3.3, 5.2	1.6, 5.6	3.0, 14.0	1.6, 14.0
DP VCN (weighted average per subject; c/dg)^b									
n	3	7	2	36	45	2	12	38	50
Median	0.75	0.60	3.01	3.75	3.40	4.05	0.70	3.79	3.39
Min, Max	0.7, 1.2	0.5, 1.3	2.3, 3.8	2.3, 5.7	0.5, 5.7	4.0, 4.1	0.5, 3.8	2.3, 5.7	0.5, 5.7
DP %LVV+ Cells (weighted average per subject; %)^b									
n	NA	6	2	36	44	2	8	38	46
Median		27.7	77.4	80.7	80.0	85.0	29.5	81.0	80.1
Min, Max		9, 42	63, 92	63, 93	9, 93	84, 86	9, 92	63, 93	9, 93
DP VCN/ DP %LVV+ Cells (weighted average per subject; vector copies per transduced cell)^b									
n	NA	6	2	36	44	2	8	38	46
Median		2.31	3.76	4.51	4.46	4.77	2.97	4.55	4.48
Min, Max		1.8, 6.5	3.5, 4.1	3.1, 6.5	1.8, 6.5	4.7, 4.9	1.8, 6.5	3.1, 6.5	1.8, 6.5

Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

6.3 General Pharmacology

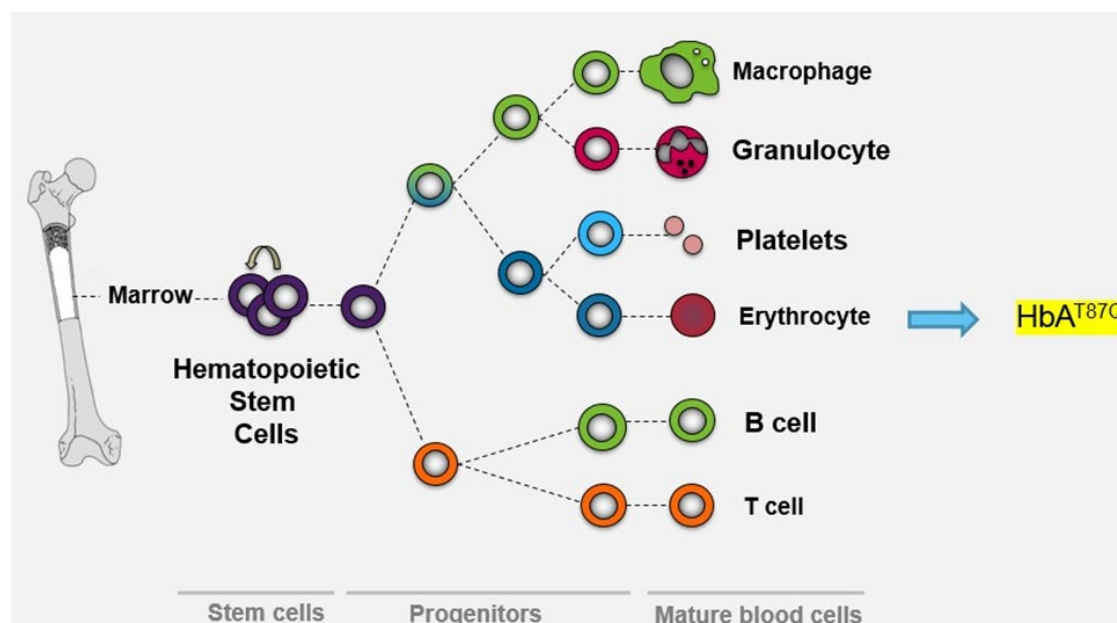
LYFGENIA (lovotibeglogene autotemcel, lovo-cel) is an autologous gene therapy consisting of hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding β^{A-T87Q} -globin. Successfully transduced patient's HSCs incorporate the β^{A-T87Q} -globin gene into their genome, and therefore their progeny contains the transgene. Figure 4 shows HSCs differentiation. The presence of vector sequences in differentiated nucleated blood cells indicates the presence of transduced cells amongst their HSC precursors. The presence of vector sequences in differentiated nucleated blood cells is detected using quantitative polymerase chain reaction (qPCR).

Although all nucleated cells derived from successfully-transduced HSCs will have vector sequences in their genome and contribute to the VCN in peripheral blood, only cells within the erythroid lineage are anticipated to produce the transcription factors required to drive expression of β^{A-T87Q} -globin because the transgene is under the transcriptional control of the erythroid lineage-specific globin LCR. Thus, only cells of the erythroid lineage are anticipated to produce transgenic β^{A-T87Q} -globin and subsequently contain HbA^{T87Q} that results from the combination of endogenous α -globin with transgenic β^{A-T87Q} -globin.

After LYFGENIA infusion, the transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce red blood cells containing biologically active β^{A-T87Q} -globin that will combine with α -globin to produce functional Hb containing β^{A-T87Q} -globin (HbA^{T87Q}). β^{A-T87Q} -globin can be distinguished from wildtype β^A -globin and from β^S -globin through reverse-phase high-performance liquid chromatography (RPHPLC) or ultra-high performance liquid chromatography (UPLC). HbA^{T87Q} has similar oxygen-binding affinity and oxygen hemoglobin dissociation curve to wild type HbA, reduces intracellular and total hemoglobin S (HbS) levels, and is designed to sterically inhibit polymerization of HbS thereby limiting the sickling of red blood cells.

Based on the nature of LYFGENIA (lovo-cel), conventional studies on pharmacokinetics, absorption, distribution, metabolism, and elimination cannot be used to monitor the presence of the drug product. To evaluate the delivery and persistence of LYFGENIA (lovo-cel), pharmacodynamic (PD) parameters were measured to detect the presence of integrated proviral sequences and the expression of transgene in differentiated cells. In addition, LYFGENIA (lovo-cel) is an autologous gene therapy consisting of HSCs that have been genetically modified ex vivo and is intended as a single dose IV infusion. The product dosing characteristics were also evaluated for their impacts on PD and clinical outcomes.

Figure 4. Hematopoietic Stem Cell Differentiation



Abbrev.: CD, cluster of differentiation; HbA^{T87Q}, HbA that contains β^{A-T87Q} -globin; HSC, hematopoietic stem cell
 Note: HSCs give rise to both myeloid and lymphoid progenitors. A common myeloid progenitor gives rise to myeloblasts (and ultimately macrophages and granulocytes [including neutrophils]), as well as erythroid cells (and ultimately erythrocytes) and megakaryotes (and ultimately platelets). A common lymphoid progenitor gives rise to T cells, B cells, and natural killer cells (which can be further identified by their CD surface antigens).

Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

6.4 Pharmacodynamics of LYFGENIA

Successful treatment with LYFGENIA requires transgene expression (β^{A-T87Q} -globin production) in the appropriate target cell population (cells of the erythroid lineage). Transgene expression requires successful transduction of HSCs, engraftment of those transduced HSCs in the subject, differentiation of transduced HSCs with subsequent erythroid compartment reconstitution, transcription and translation of the LVV-inserted transgene in cells of the erythroid lineage, and ultimately the ability of β^{A-T87Q} -globin to stable complex with α -globin to form functional Hb (HbA^{T87Q}) in mature RBCs.

Pharmacodynamic evaluation of LYFGENIA includes measurement of following PD parameters: lentiviral vector copy number (VCN), β^{A-T87Q} -globin production (HbA^{T87Q}) expression, hemoglobin fractions, and ratio of α -globin to β -like-globins in peripheral blood over time. In addition, the relationship between drug product dose characteristics and PD parameters was evaluated for LYFGENIA, an autologous gene therapy consisting of HSCs that have been genetically modified ex vivo and is intended for a single dose IV infusion.

The data cutoff date for interim analysis of Study HGB-206 is August 11, 2023. On July 26, 2023, the Applicant submitted updated data with data cutoff date on February 13, 2023. Therefore, the PD results were generally updated with the updated datasets.

6.4.1 Lentiviral Vector Copy Number in Peripheral Blood (PB VCN)

PB VCN levels were measured from Month 1 through last follow-up using qPCR method. After infusion of LYFGENIA, PB VCN levels increased rapidly over the first few months before reaching a plateau. At Month 6, the median (min, max) PB VCN level of DP2a product was 1.5 (0.6, 4.6) vc/dg (N=34). PB VCN levels generally remained stable as of the data cut-off date for all studies. High inter-subject variability of PB VCN kinetic profiles was observed (Table 4).

Table 4. Summary of Vector Copy Number in Peripheral Blood (PB VCN)

Parameter Visit	DP0/1/2 (N=12)	DP2a (N=38)	SCD (N=50)
Weighted average of DP VCN (c/dg)^a			
n	12	38	50
Median	0.70	3.79	3.39
Min, Max	0.5, 3.8	2.3, 5.7	0.5, 5.7
PB VCN (c/dg)			
Month 6			
n	12	34	46
Median	0.159	1.513	1.350
Min, Max	0.051, 2.470	0.553, 4.642	0.051, 4.642
Month 24			
n	12	29	41
Median	0.170	1.317	1.162
Min, Max	0.052, 3.047	0.406, 3.588	0.052, 3.588
Month 36			
n	11	13	24
Median	0.108	1.156	0.992
Min, Max	0.054, 2.424	0.547, 3.214	0.054, 3.214
Month 48			
n	10	6	16
Median	0.190	1.820	0.851

Min, Max	0.056, 2.738	0.940, 3.329	0.056, 3.329
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Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies (data cutoff date: August 11, 2023)

PB VCN Levels in Specific Populations

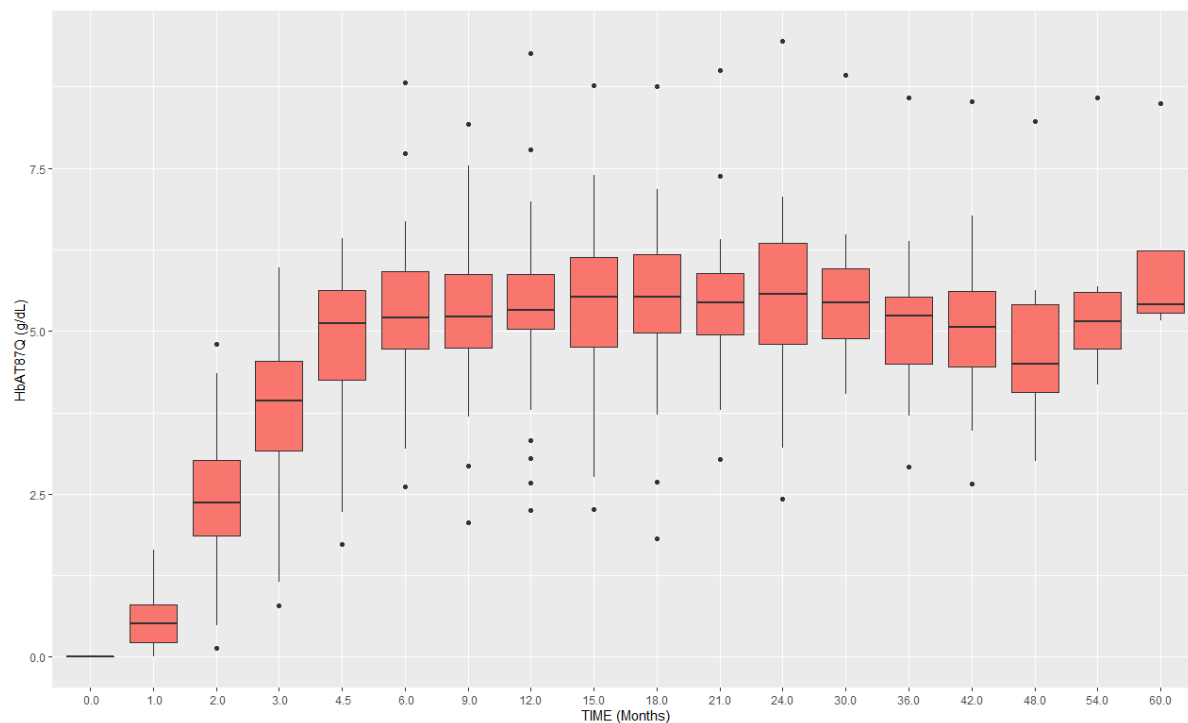
The potential impact of intrinsic factors on PB VCN levels were evaluated using population PD modeling analysis with data from Studies HGB-205, HGB-206, HGB-210 and LTF-307. The following intrinsic factors were assessed: genotype, age at baseline, race, sex, and weight at baseline. These intrinsic factors were not significant covariates and did not impact the steady-state levels and of PB VCN. Race, sex, and weight at baseline did not significantly affect time to steady-state for PB VCN either. Two subjects with “ α -thalassemia trait” (α -globin genotype: $-\alpha 3.7/-\alpha 3.7$) had higher PB VCN compared to other subjects (α -globin genotype: $\alpha\alpha/\alpha\alpha$ and $\alpha\alpha/-\alpha 3.7$). However, due to very limited sample size, no definitive conclusion can be made. Results from popPD analysis suggested a minor impact of age on the time to 90% steady-state PB VCN with ≤ 1.7 months difference between the youngest (12 years old) and oldest (38 years old) simulated. Considering that PB VCN levels were generally stable after achieving steady-state around Month 6, the impact of age was minimal.

6.4.2 HbA^{T87Q} in Peripheral Blood (PB HbA^{T87Q})

After infusion of LYFGENIA, transduced CD34⁺ HSCs engraft in the bone marrow and differentiate to produce red blood cells (RBCs) expressing biologically active β^{A-T87Q} -globin. HbA^{T87Q} is then formed through the combination of 2 α -globin subunits and 2 β^{A-T87Q} -globin subunits. Levels of HbA^{T87Q} in peripheral blood is measured using RP-HPLC.

Figure 5 and Table 5 and show the kinetic profiles of HbA^{T87Q}. HbA^{T87Q} generally increased steadily after administration of LYFGENIA, and stabilized by approximately Month 6 post-infusion. In Study HGB-206 Group C, at Month 6, the median (min, max) level of HbA^{T87Q} was 5.2 (2.6, 8.8) g/dL (N=33) and remained durable at Month 24 with median (min, max) levels of 5.5 (2.4, 9.4) g/dL (N=34). HbA^{T87Q} comprised a median (min, max) 45.7 (26.9, 63.2) (N = 34) percent of total non-transfused Hb at Month 24. Expression of HbA^{T87Q} continued to remain durable through Month 48 (N = 10), demonstrating sustained expression of the β^{A-T87Q} protein derived from irreversible integration of the β^{A-T87Q} -globin gene into long-term hematopoietic stem cells (HSCs). High inter-subject variability was observed for HbA^{T87Q} levels at any specific time point.

Figure 5. HbA^{T87Q} over time in peripheral blood (Study HGB-206 Group C)



Source: Reviewer.

HbA^{T87Q} Levels in Specific Populations

The potential impact of intrinsic factors on HbA^{T87Q} levels were evaluated using population PD modeling analysis with data from Studies HGB-205, HGB-206, HGB-210, and LFT-307. The presence of α -globin gene deletions with SCD has been shown to be associated with lower mean RBC volumes and lower Hb levels. In Study HGB206 Group C, no remarkable differences in steady state HbA^{T87Q} levels were observed among different genotype subgroups ($\alpha\alpha/\alpha\alpha$, $\alpha\alpha/-\alpha3.7$, and $-\alpha3.7/-\alpha3.7$). The HbA^{T87Q} levels at Month 6 in the two subjects with “ α -thalassemia trait” (α -globin genotype: $-\alpha3.7/-\alpha3.7$) were relatively low, but within the range of other subjects (α -globin genotype: $\alpha\alpha/\alpha\alpha$ and $\alpha\alpha/-\alpha3.7$). Based on popPD modeling analysis, subjects with the $-\alpha3.7/-\alpha3.7$ genotype were predicted to have a 1.91-fold longer time to 90% steady-state compared to subjects with either the $\alpha\alpha/\alpha\alpha$ or $\alpha\alpha/-\alpha3.7$ genotypes. However, this result should be interpreted with caution as there were only two subjects with the $-\alpha3.7/-\alpha3.7$ genotype in the analysis.

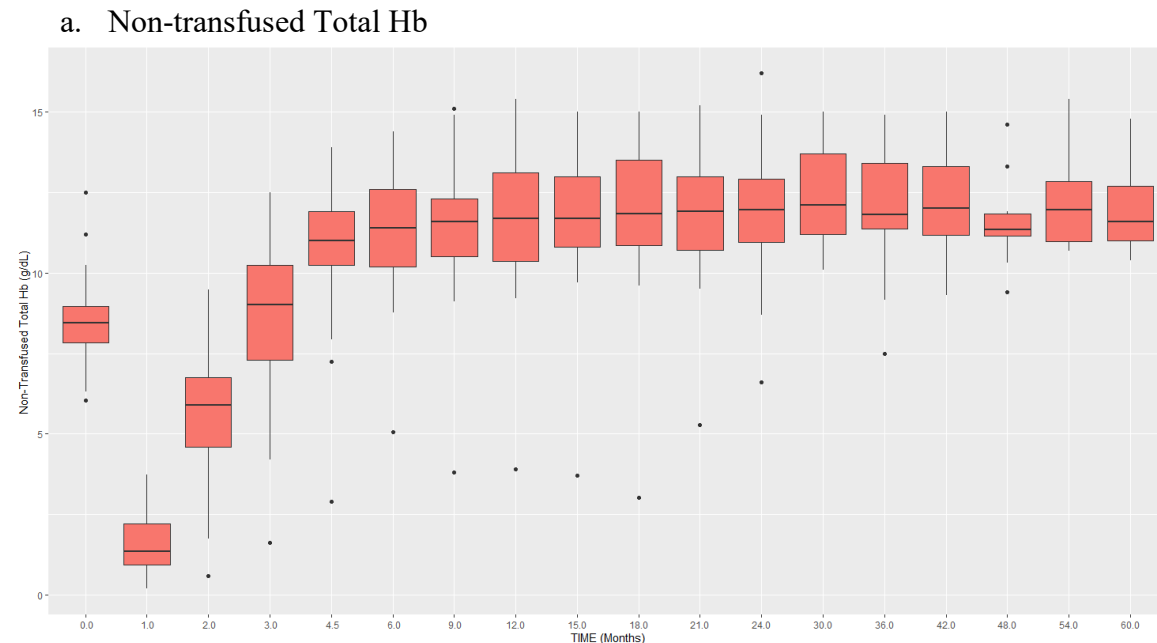
6.4.3 Hemoglobin Fractions in Peripheral Blood

The total non-transfused total Hb and Hb fractions over time were evaluated. HbA^{T87Q} and HbS are major contributors of total Hb. Table 5 summarizes levels of non-transfused total Hb and Hb fractions over time.

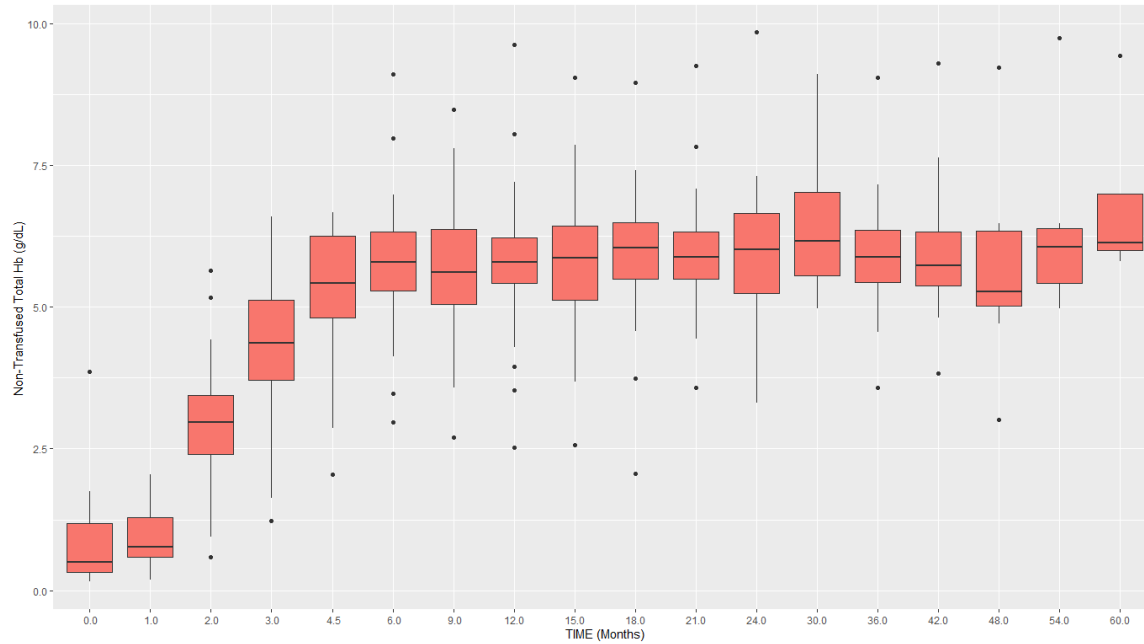
Non-transfused Total Hb: At Month 6 post-infusion of LYFGENIA, the median (min, max) non-transfused total Hb levels were 11.40 (5.06 14.40) g/dL. Non-transfused total Hb levels remained durable at Month 24 with median (min, max) level of 11.95 (6.60, 16.20) g/dL (Figure 6).

Non-transfused HbS: after the initial increase during the hematopoietic system reconstitution, the median levels of non-transfused HbS stabilized around 6 months after infusion of LYFGENIA. The median (min, max) values of HbS were 5.79 (2.97, 9.11) g/dL and 6.01 (3.31, 9.84) g/dL for Month 6 and 24 respectively. The levels of non-transfused HbS were maintained during the follow-up period (Figure 6).

Figure 6. Levels of Non-transfused total Hb and Non-transfused HbS over time in peripheral blood



b. Non-transfused HbS



Source: Reviewer.

Table 5. Summary of Hemoglobin Fractions (Study HGB-206 Group C)

	HbA187Q (g/dL)	HbS (g/dL)	HbA (g/dL)	HbA2 (g/dL)	HbF (g/dL)	Non-Transfused HbS (g/dL)	Non-Transfused Total Hb (g/dL)
Month 6							
n	33	33	33	33	33	33	33
Median	5.21	5.77	0.00	0.23	0.17	5.79	11.40
Min, Max	2.61, 8.81	1.59, 7.34	0.00, 1.44	0.00, 0.67	0.00, 1.07	2.97, 9.11	5.06, 14.40
Month 12							
n	36	36	36	36	36	36	36
Median	5.33	5.89	0.00	0.23	0.10	5.80	11.70
Min, Max	2.25, 9.26	1.39, 8.45	0.00, 5.09	0.00, 0.79	0.00, 0.68	2.53, 9.63	3.91, 15.40
Month 18							
n	32	32	32	32	32	32	32
Median	5.52	5.89	0.00	0.27	0.13	6.04	11.85
Min, Max	1.81, 8.76	0.96, 7.90	0.00, 7.28	0.16, 0.61	0.00, 0.81	2.06, 8.96	3.02, 15.00
Month 24							
n	40	40	40	40	40	40	40
Median	5.57	5.88	0.00	0.27	0.08	6.01	11.95
Min, Max	2.42, 9.44	1.92, 7.96	0.00, 1.80	0.16, 0.61	0.00, 1.13	3.31, 9.84	6.60, 16.20
Month 36							
n	18	18	18	18	18	18	18
Median	5.23	6.04	0.00	0.28	0.57	5.88	11.80
Min, Max	2.91, 8.58	3.91, 7.74	0.00, 6.72	0.19, 0.35	0.06, 0.89	3.58, 9.05	7.49, 14.90

Month 48							
n	10	10	10	10	10	10	10
Median	4.49	6.31	0.00	0.25	0.53	5.27	11.35
Min, Max	3.01, 8.22	4.88, 6.84	0.00, 0.00	0.00, 0.35	0.00, 1.35	3.01, 9.22	9.40, 14.61
Month 60							
n	4	4	4	4	4	4	4
Median	5.40	5.18	0.00	0.26	0.45	6.13	11.60
Min, Max	5.16, 8.50	4.59, 5.94	0.00, 0.00	0.24, 0.30	0.41, 0.68	5.80, 9.44	10.39, 14.79

Source: Reviewer.

6.4.4 Ratio of α -Globin/All β -like Globins

Normal globin chain production and metabolism keeps the ration of α -chain to non- α -chain approximately equal. Relative globin levels in soluble hemolysates were measured by RP-HPLC or UPLC. The median (min, max) ratio of α -globin/all β -like globins was 1.09 (0.99, 1.55) (n=33) at Month 6 post-infusion of LYFGENIA and the ratios were generally maintained during the study. At Month 24, the median (min, max) ratio of α -globin/all β -like globins was 1.07 (1.01, 1.67) (n=40).

In addition to the β S-globin mutation, some subjects treated with lovo-cel had mutations that affect the number of copies of α -globin genes. Normally, people have 4 normal α -globin genes (2 copies of *HBA1*, 2 copies of *HBA2*), with *HBA2* genes contributing approximately 60% of the α -globin protein. Inactivation of a single α -globin gene copy is called “silent α -thalassemia” whereas inactivation of 2 copies is called “ α -thalassemia- trait”. The presence of α -globin gene deletions with SCD has been shown to be associated with lower mean RBC volumes and lower Hb levels. The most common α -globin gene deletion is the 3.7 kb rightward deletion ($-\alpha 3.7$), which is caused by the breakage of DNA molecules in the α globin genes (*HBA2* and *HBA1*) region and rejoining of the broken ends leaving a single functional gene. The $-\alpha 3.7$ mutation is common in African Americans, and approximately 37% of the *DP2a Pool* had at least one copy of the $-\alpha 3.7$ mutation, and 2 (5.3%) subjects had 2 copies of this mutation. No substantive differences of α -globin/all β -like globins were observed among different α -globin genotypes (Table 6).

Table 6. Ratio of α -globin/all β -like Globins at Month 6 by Genotype (Study HGB-206 Group C)

	Ratios of α -Globin/All β -like Globins
$\alpha\alpha/\alpha\alpha$	
n	22
Median	1.09
Min, Max	1.01, 1.55
$\alpha\alpha/-\alpha 3.7$	
n	9
Median	1.06
Min, Max	1.03, 1.45
$-\alpha 3.7/-\alpha 3.7$	

n	2
Median	1.01
Min, Max	0.99, 1.03

Source: Reviewer.

6.5 LYFGENIA Drug Product Dosing Characteristics and Pharmacodynamic Responses, Clinical Outcomes

LYFGENIA is an autologous gene therapy consisting of HSCs that have been genetically modified ex vivo and is intended for a single dose IV infusion. Considering the heterogenicity of LYFGENIA product composition and one-time treatment dosing regimen, the product dosing characteristics were also evaluated for their impacts on PD and clinical outcomes.

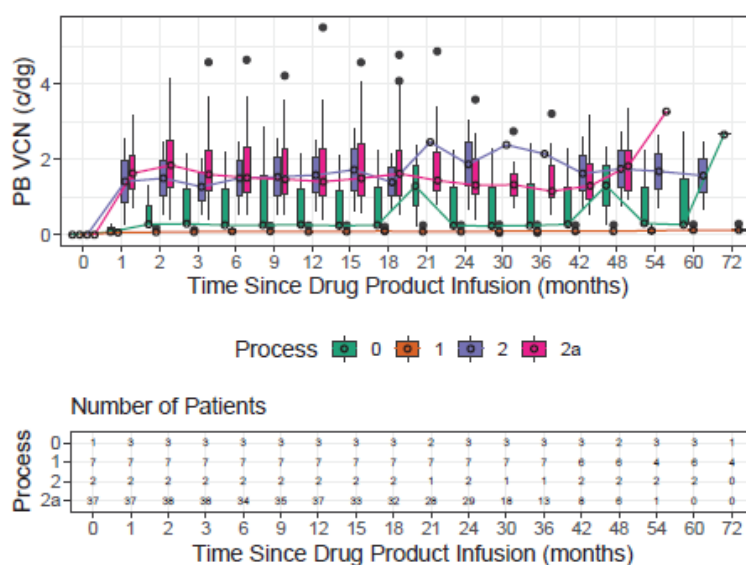
6.5.1 Drug Product Dosing Characteristics and Pharmacodynamic Responses

As mentioned in Section 6.2, different manufacturing process was used in the clinical development of LYFGENIA. The PD responses of LYFGENIA from different manufacturing processes were assessed. As shown in Figure 7, PB VCN was lower in patients that received DP manufactured via Process 0 and 1 compared to those that received DP manufactured via Process 2 and 2a.

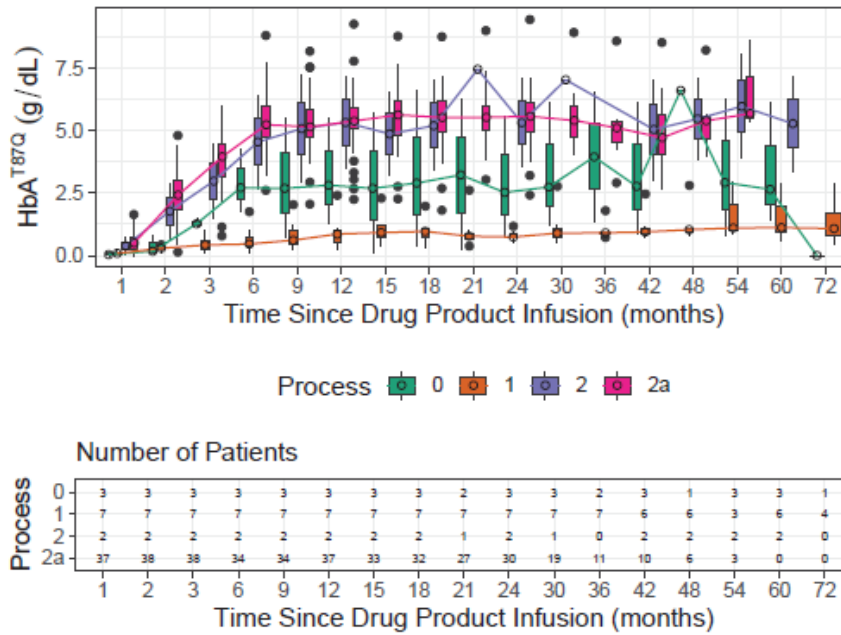
Subjects received DP manufactured with sLVV (n=6) had a higher PB VCN compared to those that received drug product manufactured with aLVV (n=29) or with a mixed LVV type (n=3). Additionally, sLVV appeared to result in a marginally longer time to maximum PB VCN compared to aLVV or mixed LVV type. A similar trend was seen when the analysis was restricted to patients that received DP manufactured by Process 2a (Figure 8).

Figure 7. PB VCN Profile by Manufacturing Process

a. PB VCN



c. HbA^{T87Q}

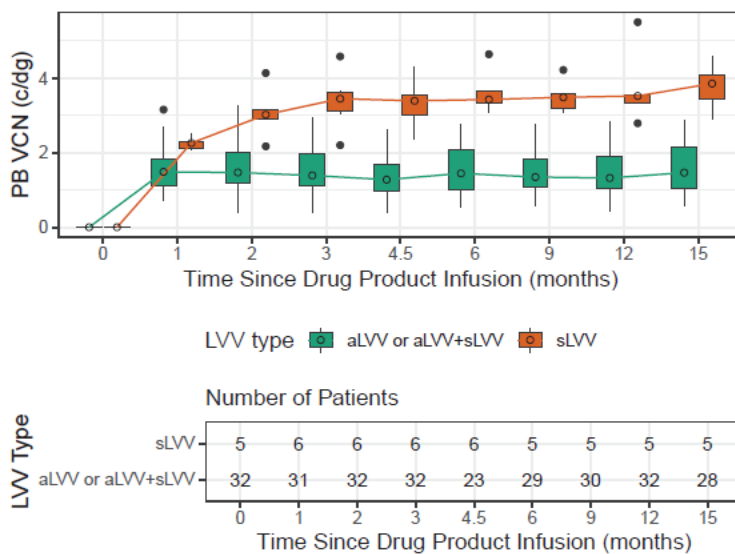


Source: Applicant. Module 5, Section 5.3.5.3. Population Pharmacodynamic Analysis Report.

As shown in Figure 6, HbAT87Q was lower in patients that received drug product manufactured via Process 0 and 1 compared to those that received drug product manufactured via Process 2 and 2a.

Figure 8. PB VCN Profile by LVV Type (DP2a Pool)

a. PB VCN



Source: Applicant. Module 5, Section 5.3.5.3. Population Pharmacodynamic Analysis Report.

Pearson correlations analysis was conducted to investigate the relationships between specific drug product dosing characteristics (DP VCN, DP %LVV+ Cells, and Total Cell Dose) and PD responses (PB VCN and HbA^{T87Q}) and results are shown in Table 7.

Table 7. Relationships between Dosing Characteristics and Pharmacodynamic Parameters (DP2a Pool)

Dosing Characteristic X-axis (Independent)	PD Parameter Y-axis (Dependent)	Correlation Coefficient	p-value
Weighted average DP VCN	PB VCN at Month 6	0.3905	0.0224 ^a
	%HbA ^{T87Q} at Month 6 ^b	0.2665	0.1277
Weighted average DP %LVV+ Cells	PB VCN at Month 6	0.5398	0.0010 ^a
	%HbA ^{T87Q} at Month 6 ^b	0.5317	0.0012 ^a
Total Cell Dose	%HbA ^{T87Q} at Month 6 ^b	-0.1688	0.3558

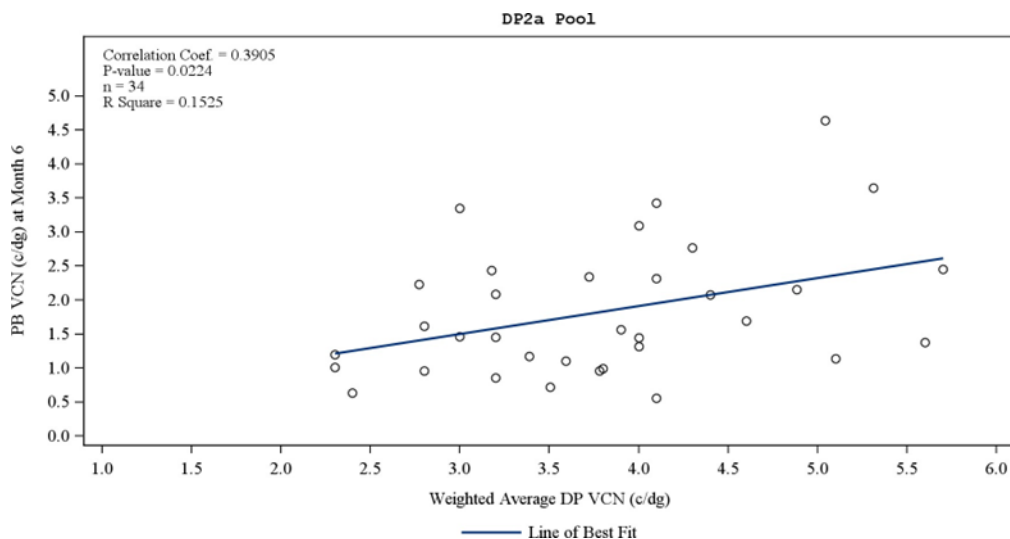
Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

6.5.1.1 DP VCN and DP %LVV+ Cells versus PB VCN at Month 6

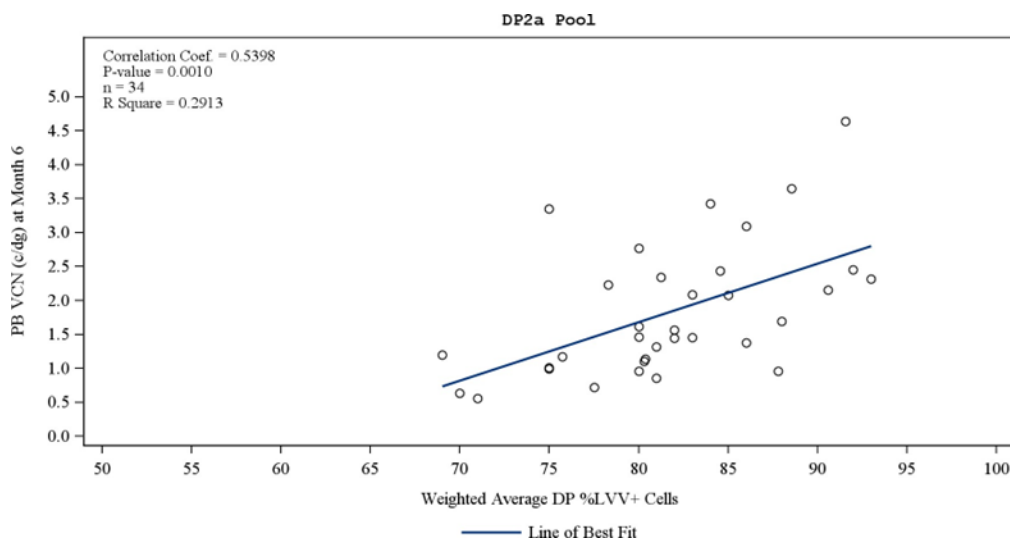
As shown in Figure 9, both DP VCN (correlation coefficient $r = 3905$, p-value = 0.0224, $n = 34$) and DP %LVV+ Cells (correlation coefficient $r = 5398$, p-value = 0.0010, $n = 34$) correlated with PB VCN, with the latter showing the stronger correlation.

Figure 9. DP VCN and DP %LVV+ Cells versus PB VCN at Month 6

a. DP VCN



b. DP %LVV+ Cells



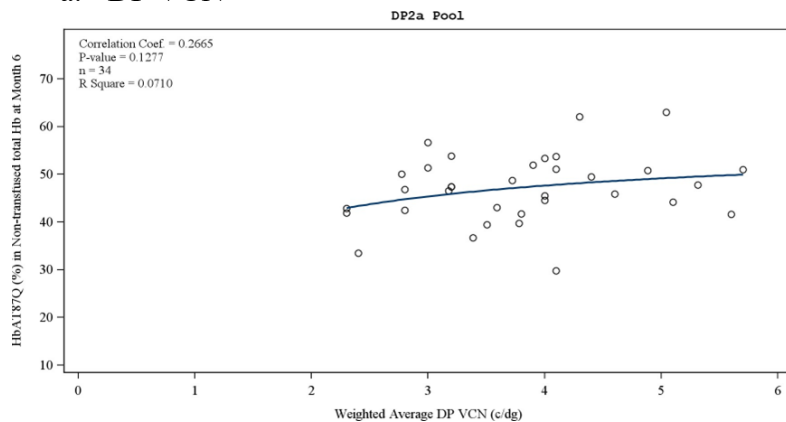
Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies.

6.5.1.2 DP VCN and DP %LVV+ Cells versus HbA^{T87Q} at Month 6

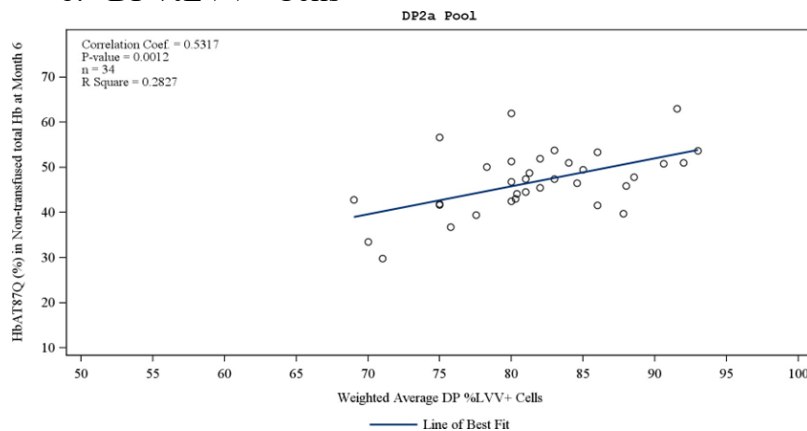
There's no significant correlation observed between DP VCN and %HbA^{T87Q} in non-transfused Hb at Month 6 (correlation coefficient $r = 0.2665$, $p\text{-value} = 0.1277$, $n = 34$). DP %LVV+ Cells showed a significant correlation (correlation coefficient $r = 0.5317$, $p\text{-value} = 0.0012$, $n = 34$) (Figure 10). This was in line with the stronger correlation between DP %LVV+ Cells and PB VCN. The optimization of drug product manufacturing and regulation of globin levels in erythroid cells results in the majority of subjects producing Hb^{AT87Q} over a narrow percentage range (on a plateau).

Figure 10. DP VCN and DP %LVV+ Cells versus %HbA^{T87Q} at Month 6 (DP2a Pool)

a. DP VCN



b. DP %LVV+ Cells

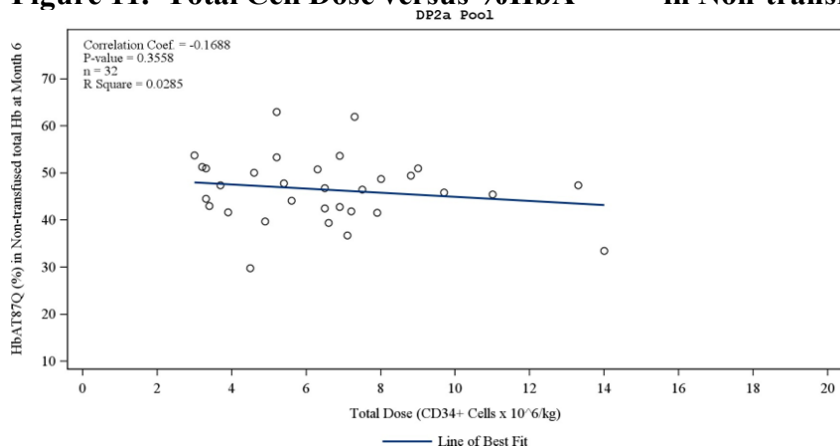


Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies.

6.5.1.3 Total CD34+ Cell Dose versus HbA^{T87Q} at Month 6

As shown in Figure 11, no correlation was observed between total cell dose of LYFGENIA and %HbA^{T87Q} in peripheral blood at Month 6.

Figure 11. Total Cell Dose versus %HbA^{T87Q} in Non-transfused Total Hb at Month 6



Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies.

6.5.2 Drug Product Dosing Characteristics and Clinical Outcomes

6.5.2.1 Drug Product Dosing Characteristics and Engraftment

The relationship between LYFGENIA dosing characteristics (total cell dose, DP VCN and DP %LVV+ Cells) and engraftment (Day of engraftment for either neutrophil or platelets) was evaluated.

All subjects treated with LYFGENIA achieved successful neutrophil engraftment within 42 days after LYFGENIA infusion (median: 21 days, range: 12 – 38 days). The median (min, max) time to achieve platelet engraftment was 39.0 (19, 136) days.

No correlation was observed between cell dose and either neutrophil or platelet engraftment, indicating that even the lowest cell doses evaluated to were adequate for effective reconstitution of HSCs in treated subjects for DP2a Pool.

The relationship between DP VCN or DP %LVV+ Cells and the Day of platelet engraftment were also evaluated. No meaningful relationships were observed: for DP2a Pool, for DP VCN the correlation coefficient $r = 0.1962$, with $p\text{-value} = 0.2379$; for DP %LVV+ Cells, correlation coefficient $r = 0.2907$, $p\text{-value} = 0.0767$.

6.5.2.2 Drug Product Dosing Characteristics and Complete Resolution of Vaso-occlusive Events (VOE-CR)

No significant correlation was observed between dosing characteristics and complete resolution of vaso-occlusive events (VOE-CR) in DP2a Pool.

6.6 Pharmacodynamic Responses and Clinical Outcomes

6.6.1 Relationships between Pharmacodynamic Parameters

Correlative analysis was performed to assess the relationships among different PD parameters. The results are shown in Table 8.

Table 8. Relationships between Pharmacodynamic Parameters

X-axis (Independent)	Y-axis (Dependent)	Population ^a	Correlation Coefficient	p-value
PB VCN at M6	PB VCN at M12	<i>SCD</i> <i>DP2a</i>	r = 0.9853 r = 0.9764	p = <0.0001 p = <0.0001
	PB VCN at M24	<i>SCD</i> <i>DP2a</i>	r = 0.9650 r = 0.9255	p = <0.0001 p = <0.0001
%HbA ^{T87Q} in Non-transfused Hb at M6	%HbA ^{T87Q} in Non-transfused Hb at M12	<i>SCD</i> <i>DP2a</i>	r = 0.9851 r = 0.9335	p = <0.0001 p = <0.0001
	%HbA ^{T87Q} in Non-transfused Hb at M24	<i>SCD</i> <i>DP2a</i>	r = 0.9654 r = 0.8584	p = <0.0001 p = <0.0001
Non-transfused Total Hb at M6	Non-transfused Total Hb at M12	<i>SCD</i> <i>DP2a</i>	r = 0.9079 r = 0.9184	p = <0.0001 p = <0.0001
	Non-transfused Total Hb at M24	<i>SCD</i> <i>DP2a</i>	r = 0.8144 r = 0.8065	p = <0.0001 p = <0.0001
Non-transfused HbS at M6	Non-HbS at M12	<i>SCD</i> <i>DP2a</i>	r = 0.9573 r = 0.9288	p = <0.0001 p = <0.0001
	Non-HbS at M24	<i>All TDT</i> <i>All Phase 3</i>	r = 0.9342 r = 0.8464	p = <0.0001 p = <0.0001
PB VCN at M6	PB %LVV+ Cells at M6	<i>SCD</i> <i>DP2a</i>	r = 0.7036 r = 0.7036	p = 0.0107 p = 0.0107
PB VCN at M6	%HbA ^{T87Q} in Non-transfused Hb at M6	<i>SCD</i> <i>DP2a</i>	r = 0.9547 r = 0.8489	p = <0.0001 p = <0.0001
PB %LVV+ Cells at M6	%HbA ^{T87Q} in Non-transfused Hb at M6	<i>SCD</i> <i>DP2a</i>	r = 0.7433 r = 0.7433	p = 0.0056 p = 0.0056
HbA ^{T87Q} at M6	%HbA ^{T87Q} in Non-transfused Hb at M6	<i>SCD</i> <i>DP2a</i>	r = 0.9008 r = 0.5642	p = <0.0001 p = 0.0005

Non-transfused Total Hb at M6	%HbA ^{T87Q} at M6	SCD DP2a	r = 0.4549 r = -0.0464	p = 0.0015 p = 0.7944
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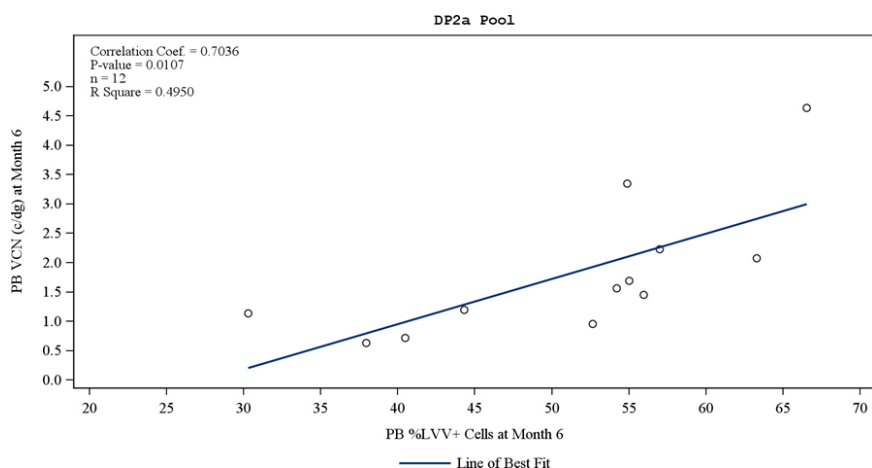
After infusion of LYFGENIA, PD biomarkers, such as PB VCN and HbA^{T87Q}, non-transfused HbS, non-transfused total Hb generally increased steadily to reach a plateau. For above mentioned PD biomarkers, correlation was observed between Month 6 and later time points, such as Month 12 and Month 24 levels in both SCD and DP2a Pools.

There was a strong correlation between HbAT87Q and %HbAT87Q in total non-transfused Hb for both SCD and DP2a Pools.

6.6.1.1 Relationships between PB VCN and PB %LVV+ Cells at Month 6

PB VCN and PB %LVV+ Cells both measure characteristics of the peripheral blood cells that reflect the relative contribution of transduced HSC to peripheral blood cells. Figure 12 shows the correlation between PB VCN and PB %LVV+ Cells at Month 6.

Figure 12. Correlation of PB VCN at Month 6 versus PB %LVV+ Cells at Month 6 (DP2a Pool)



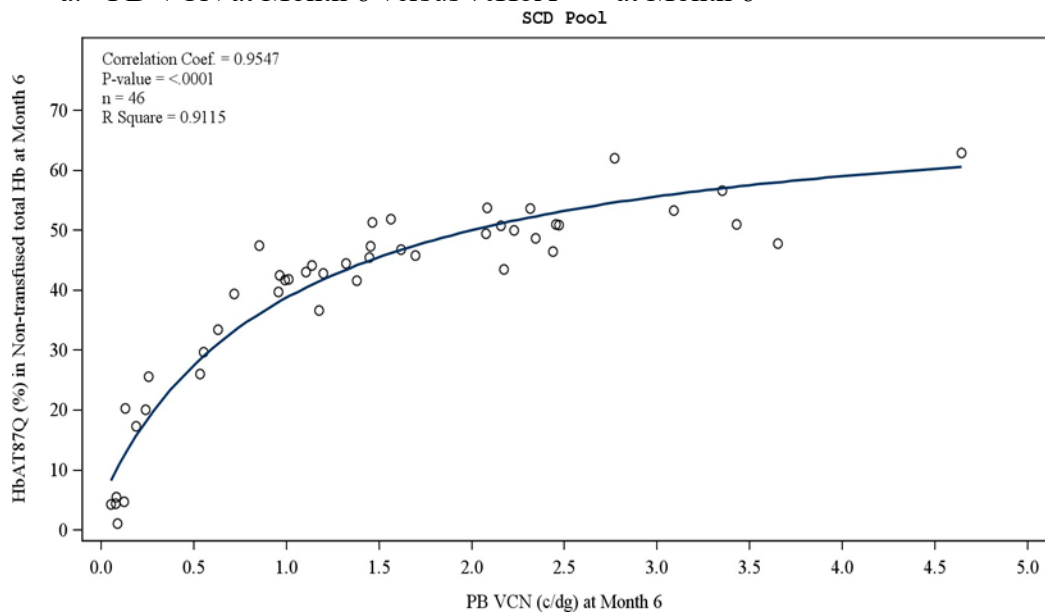
Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

6.6.1.2 Relationships between PB VCN and PB %LVV+ Cells versus %HbA^{T87Q} in non-transfused Hb at Month 6

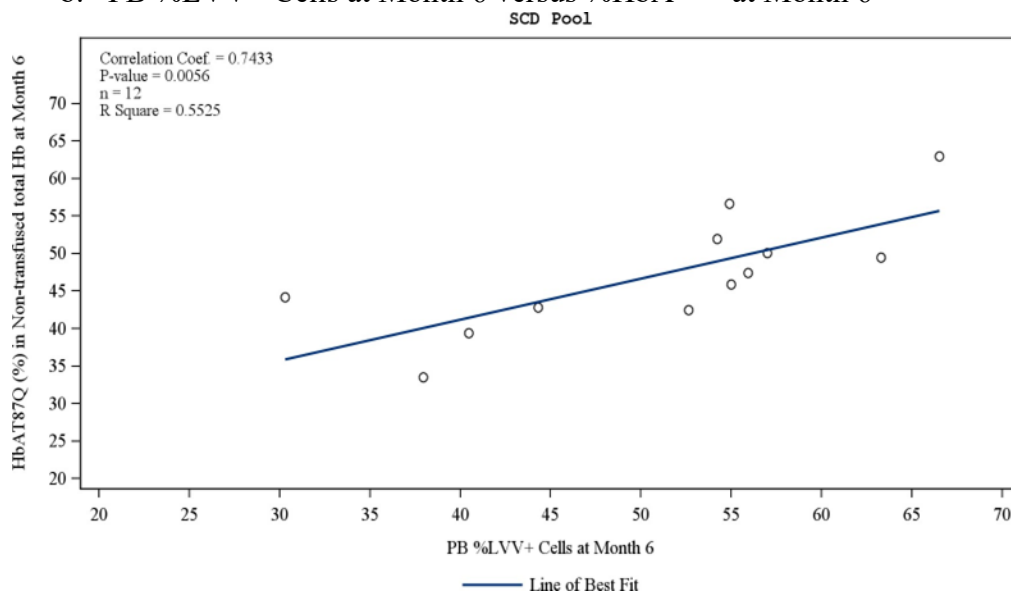
Figure 13 depicts the relationship between PB VCN and PB %LVV+ Cells versus %HbA^{T87Q} at Month 6. %HbA^{T87Q} increased quickly with PB VCN at lower PB VCN levels, followed by a %HbA^{T87Q} plateau at higher PB VCN levels. This reflects the underlying regulation of the β -globin levels within erythroid cells. Significant correlation was observed between PB %LVV+ Cells and %HbA^{T87Q} at Month 6.

Figure 13. Relationship Between PB VCN and PB %LVV+ Cells and %HbA^{T87Q} at Month 6

a. PB VCN at Month 6 versus %HbA^{T87Q} at Month 6



b. PB %LVV+ Cells at Month 6 versus %HbA^{T87Q} at Month 6



Source: Applicant. Module 2, Section 2.7.2. Summary of Clinical Pharmacology Studies

6.6.2 PD responses and Clinical Outcome – Complete Resolution of Vaso-Occlusion Events (VOE-CR)

The primary efficacy endpoint in Study HGB-206 was complete resolution of vaso-occlusive events (VOE-CR) between 6 months and 18 months after drug product infusion. The relationships between PD responses (HbA^{T87Q} and non-transfused total Hb) and VOE-CR were assessed.

HbAT87Q and VOE-CR

In the evaluable subjects, 3 of 14 (21%) subjects with lower than median level of HbA^{T87Q} (5.3 g/dL) did not achieve VOE-CR. One of 15 (7%) subjects with higher than and median levels of HbA^{T87Q} did not achieve VOE-CR (Table 9).

Non-transfused Total Hb and VOE-CR

In the evaluable subjects, 4 of 14 (29%) subjects with lower than median level of non-transduced total Hb (11.4 g/dL) did not achieve VOE-CR. All 15 subjects with higher than and median levels of non-transduced total Hb achieved VOE-CR (Table 9).

Table 9. Pharmacodynamic Responses (HbA^{T87Q} and Non-transfused Total Hb) at Month 6 versus VOE-CR

a. HbA^{T87Q} at Month 6 versus VOE-CR

	N	Achieved VOE- CR	Did Not Achieve VOE-CR
< Median HbAT87Q level (5.3 g/dL) at Month 6	14	11	3
≥ Median HbAT87Q level (5.3 g/dL) at Month 6	15	14	1
Total evaluable subjects	29	25	4

b. Non-transfused Total Hb at Month 6 versus VOE-CR

	N	Achieved VOE- CR	Did Not Achieve VOE-CR
< Median Non-transfused Total Hb Level (11.4 g/dL) at Month 6	14	10	4
≥ Median Non-transfused Total Hb Level (11.4 g/dL) at Month 6	15	15	0
Total evaluable subjects	29	25	4

Source: Reviewer.

6.7 Clinical Pharmacology Conclusions

- Subjects in Study 206 Group C (N=36) received a median (min, max) dose of LYFGENIA of $6.4 (3.0, 14.0) \times 10^6$ CD34+ cells/kg.
- After infusion of LYFGENIA, lentiviral vector copy number in peripheral blood (PB VCN) levels increased rapidly over the first few months before reaching a plateau. At Month 6, the median (min, max) PB VCN level of DP2a product was 1.5 (0.6, 4.6) vc/dg (N=36). PB VCN levels generally remained stable as of the data cut-off date for all studies, although high inter-subject variability of PB VCN kinetic profiles were observed.
- HbA^{T87Q} generally increased steadily after administration of LYFGENIA, and stabilized by approximately Month 6 post-infusion. At Month 6, the median (min, max) level of HbA^{T87Q} was 5.2 (2.6, 8.8) g/dL (N=33) and remained durable at Month 24 with median (min, max) levels of 5.5 (2.4, 9.4) g/dL (N=34). HbA^{T87Q} comprised a median (min, max) 45.7 (26.9, 63.2) (N = 34) percent of total non-transfused Hb at Month 24. Expression of HbA^{T87Q} continued to remain durable through Month 48 (N = 10), demonstrating sustained expression of the β^{A-T87Q} protein derived from irreversible integration of the β^{A-T87Q} -globin gene into long-term hematopoietic stem cells (HSCs).
- At Month 6 post-infusion of LYFGENIA, the median (min, max) non-transfused total Hb levels were 11.4 (5.1, 14.4) g/dL (N=33). Non-transfused total Hb levels remained durable at Month 24 with median (min, max) levels of 11.8 (6.6, 16.2) g/dL (N=34).
- The kinetic profile of HbS was similar as HbA^{T87Q}. HbS levels increased initially after administration of LYFGENIA, and stabilized by approximately Month 6 post-infusion. At Month 6, the median (min, max) level of HbS was 5.8 (1.6, 7.3) g/dL (N=33). HbS levels remained stable during the study. At Month 24, the median (min, max) HbS was 5.8 (1.9, 8.0) g/dL (N=34).
- The amount of each hemoglobin (Hb) fraction as well as the total Hb was generally stable by 6 months post-infusion of LYFGENIA. The relative percentages of HbA^{T87Q} and HbS were also stable over time.
- LYFGENIA manufactured from suspension culture (sLVV) and adherent culture (aLVV) had similar median values for DP VCN and DP %LVV+ Cells. Subjects who received sLVV had higher median PB VCN levels compared to subjects received aLVV. Similar median HbA^{T87Q} levels and key efficacy endpoint (complete resolution of vaso-occlusive event, VOE-CR) were observed between sLVV and aLVV subgroups. Due to the small sample size of sLVVgroup, the results should be interpreted with caution.
- The targeted AUC range of busulfan evaluated in clinical studies was considered adequate for myeloablation.
- Non-transfused total Hb and complete resolution of vaso-occlusive event (VOE-CR): in the evaluable subjects, 4 of 14 (29%) subjects with lower than median level of non-transduced total Hb (11.4 g/dL) did not achieve VOE-CR. All 15 subjects with higher than and median levels of non-transduced total Hb achieved VOE-CR.

- HbA^{T87Q} at Month 6 and resolution of vaso-occlusive event (VOE-CR): in the evaluable subjects, 3 of 14 (21%) subjects with lower than median level of HbA^{T87Q} (5.2 g/dL) did not achieve VOE-CR. One of 15 (7%) subjects with higher than and median levels of HbA^{T87Q} did not achieve VOE-CR.

7 APPENDIX - INDIVIDUAL STUDY

7.1 Study #1 – Study HGB-205

Study Completion: February 26, 2019

<p>Title: A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Disease and β-Thalassemia Major) by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral β^{A-T87Q}-Globin Vector (LentiGlobin BB305 Drug Product)</p>
<p>Objectives: Primary Objective: Determine the safety, tolerability, and success of engraftment with LentiGlobin BB305 Drug Product after conditioning with Busilvex[®] (busulfan IV) in subjects with severe sickle cell disease (SCD) or transfusion-dependent β-thalassemia (TDT) Secondary Objectives:</p> <ul style="list-style-type: none"> • Quantify gene transfer efficiency and expression • Evaluate β^{A-T87Q}-globin in blood • Quantify the hematopoietic chimerism resulting from treatment with LentiGlobin BB305 Drug Product (evaluate vector copy number [VCN] in blood) • Measure the effects of transplantation with LentiGlobin BB305 Drug Product on the expression of disease-specific biological parameters and clinical events, including the volume of blood transfusions for both severe SCD and TDT and, for subjects with severe SCD, the number of vaso-occlusive crises (VOCs) and acute chest syndrome (ACS) events, in each subject compared with his/her 2-year pre-treatment period
<p>Study Design This was a single-arm, single site, single dose, Phase 1/2 study in subjects with severe SCD, or TDT with 4 distinct stages: (1) screening, (2) mobilization/apheresis for autologous cell collection, (3) conditioning with busulfan, followed by drug product infusion on Day 1, and (4) follow-up until study completion at Month 24..</p>
<p>Number of Subjects: Planned: 7 subjects with drug product; Enrolled and treated: 4 subjects with TDT (aged 16, 17, 18, and 19 years) and 3 subjects with SCD (aged 13, 16, and 21 years)</p>
<p>Main Criteria for Inclusion: Subjects between 5 and 35 years of age, inclusive, with severe SCD or TDT, eligible for allogeneic HSCT (allo-HSCT) but without a suitable, willing, 10/10 matched human leukocyte antigen (HLA)-identical sibling donor. Subjects with TDT must be stable and maintained on an appropriate iron chelation regimen and have a history of ≥ 100 mL/kg/year of packed red blood cells (pRBCs) in each of the 2 years prior to enrollment. Subjects with severe SCD must have failed to achieve adequate clinical benefit following hydroxyurea treatment and have 1 or more of the following poor prognostic risk factors: recurrent vaso-occlusive crises (VOC), significant cerebral abnormality on magnetic resonance imaging (MRI), stroke without any severe cognitive disability, osteonecrosis of 2 or more joints, anti-erythrocyte alloimmunization (>2 antibodies), cardiomyopathy documented by Doppler echocardiography, acute chest syndrome (at least 2 episodes). Subjects with severe SCD and cerebral vasculopathy (defined by overt stroke; abnormal transcranial Doppler [> 170 cm/sec]; or occlusion or stenosis in the polygon of Willis; or presence of Moyamoya disease) could be</p>

enrolled only with approval by the Comité de Surveillance after review of safety and efficacy data from ≥ 2 SCD subjects without cerebral vasculopathy treated with LentiGlobin BB305 Drug Product; no subjects meeting this requirement were enrolled.

Study Treatments

Investigational medicinal product(s): LentiGlobin BB305, lovo-cel

Dose: $\geq 3.0 \times 10^6$ CD34+ cells/kg (8.79 to 13.6×10^6 CD34+ cells/kg) for TDT; $\geq 2.0 \times 10^6$ CD34+ cells/kg (2.96 to 5.55×10^6 CD34+ cells/kg) for SCD

Formulation: cell suspension

Route(s) of administration: intravenous (IV)

Dose regimen: single dose

Pharmacodynamic Sampling Times

Please refer to 7.5.1.

Pharmacodynamic Results:

TDT:

Parameter	1201	1202	1203	1206
Genotype	β^E/β^0	β^E/β^0	β^+/β^+	β^E/β^0
DP VCN (c/dg)	1.5	2.1	0.8	1.1
PB VCN at M24 (c/dg)	0.936	3.313	0.384	2.549
HbA ^{T87Q} at M24 (g/dL)	7.27	10.13	6.72	9.03
HbE at M24 (g/dL)	2.69	2.71	ND	2.08
HbA at M24 (g/dL)	0	0	1.00	0
HbA ₂ at M24 (g/dL)	0.39	0.36	0.56	0.27
HbF at M24 (g/dL)	0.15	0.30	0.42	0.13
Total Hb at M24 (g/dL)	10.5	13.5	8.7	11.5
Ratio of α -globin/all β -like globins	0.86	1.10	1.17	1.26

SCD:

Parameter	1204	1207	1208
Cell Dose ($\times 10^6$ CD34+ cells/kg)	5.55	4.74	2.96
DP VCN (c/dg)	1.1	0.85	0.65
PB VCN at M24 (c/dg)	2.244	0.240	0.211
HbA ^{T87Q} at M24 (g/dL)	5.57	0.74	2.53
HbS at M24 (g/dL)	6.39	2.64	5.88
HbA ₂ at M24 (g/dL)	0.23	0.22	0.26
HbF at M24 (g/dL)	0.10	0.36	1.14
Anti-sickling Hb at Month 24 (HbA ^{T87Q} , HbF, HbA ₂ ; g/dL)	5.90	1.32	3.93

%Anti-sickling Hb at M24	48.05	13.99	39.99
%HbS/total Hb at M24	51.95	28.07	60.01
%HbS/nontransfused total Hb at M24	51.95	66.74	60.01
%HbA ^{T87Q} /total Hb at M24	45.30	7.91	25.78
%HbA ^{T87Q} / nontransfused total Hb at M24	45.30	18.81	25.78
HbA ^{T87Q} /HbS Ratio at M24	0.87	0.28	0.43
Total Hb at M24 (g/dL)	12.3 (transfusion-free since Day 89)	9.4 (receiving pRBC transfusions)	9.8 (transfusion-free since Day 16)

Source: Applicant. Module 5, section 5.3 Clinical Study Reports.

7.2 Study #2 – Study HGB-206

Interim Analysis Data Cutoff Date: August 11 2022

Title: A Phase 1/2 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ stem cells Transduced Ex-Vivo with the LentiGlobin BB305 Lentiviral Vector in Subjects with Severe Sickle Cell Disease.					
Objectives:					
Primary Objectives:					
Evaluate the efficacy of treatment with lovo-cel in subjects with severe sickle cell disease (SCD).					
Secondary Objectives:					
Evaluate the safety of treatment with lovo-cel in subjects with severe SCD.					
Methodology:					
This is a non-randomized, open label, multi-site, single dose, Phase 1/2 study in approximately 50 adults and adolescents with severe SCD. Treatment was divided into 4 stages: Stage 1: screening and eligibility assessment; Stage 2: stem cell harvest, drug product manufacture and disposition; Stage 3: myeloablative conditioning and infusion of lovo-cel; Stage 4: Follow-up, through 24 months after drug product infusion.					
Number of Subjects:					
	Number of Subjects				
	Group A	Group B1	Group B2	Group C	Total
Planned to be Treated	7	1	1	Approximately 41	Approximately 50
Analyzed (ITT)	9	1	1	43	54
Abbrev.: ITT, intent-to-treat population Enrollment is completed in this study.					
Main Criteria for Inclusion/Exclusion:					
Key inclusion/exclusion criteria for all subjects:					
Subjects must have been: ≥ 12 and ≤ 50 years of age at time of consent (or assent, as applicable), with SCD					

(diagnosis with either β^S/β^S , β^S/β^0 , or β^S/β^+ genotype); and for subjects < 18 years of age, a willing, matched human leukocyte antigen (HLA)-identical sibling hematopoietic cell donor is not available.

Key additional inclusion criterion as of Protocol Version 8.0 and thereafter:

Subjects must have experienced at least 4 severe vaso-occlusive events (sVOEs) in the 24 months prior to Informed Consent and failed to achieve adequate clinical benefit following hydroxyurea (HU).

Study Treatments

Investigational medicinal product(s): LentiGlobin BB305, lovo-cel

Dose: Subjects received lovo-cel at a dose of 1.5×10^6 CD34+ cells/kg (Protocol V1.0 through V5.0) or 2.0×10^6 CD34+ cells/kg (Protocol V6.0 onward) for drug product manufactured from bone marrow, or $\geq 3.0 \times 10^6$ CD34+ cells/kg for drug product manufactured from plerixafor-mobilized cells collected by apheresis.

Formulation: cell suspension

Route(s) of administration: intravenous (IV)

Dose regimen: single dose

Pharmacodynamic Sampling Times:

Please refer to 7.5.1.

Pharmacodynamic Results:

Parameter	Statistic	Group A	Group B	Group C
DP Characteristics				
DP VCN (weighted average per subject; c/dg)	n	7	2	36
	Median	0.60	3.01	3.75
	Min, Max	0.5, 1.3	2.3, 3.8	2.3, 5.7
DP %LVV+ Cells (weighted average per subject)	n	6	2	36
	Median	27.7	77.4	80.7
	Min, Max	9, 42	63, 92	63, 93
Total Dose (CD34+ cells \times 10^6 /kg)	n	7	2	34
	Median	2.10	2.70	6.40
	Min, Max	1.6, 5.1	2.2, 3.2	3.0, 14.0
DP VCN/DP %LVV+ Cells (weighted average per subject; vector copies per transduced cell)	n	6	2	36
	Median	2.31	3.76	4.51
	Min, Max	1.8, 6.5	3.5, 4.1	3.1, 6.5
PD Parameters based on DNA Analysis				
PB VCN at M6 (c/dg)	n	7	2	32
	Median	0.089	1.502	1.458
	Min, Max	0.051, 0.189	0.533, 2.470	0.553, 4.642
PD Parameters based on Protein Expression				
HbA ^{T87Q} at Month 6 (g/dL)	n	7	2	32
	Median	0.46	4.54	5.19
	Min, Max	0.1, 1.8	2.7, 6.4	2.6, 8.8
HbS at Month 6 (g/dL)	n	7	2	32
	Median	6.84	6.02	5.79
	Min, Max	3.5, 8.1	5.9, 6.1	1.6, 7.3

All Subjects Maintained Integrated Transgene in Peripheral Blood Leukocytes

- All treated subjects for whom data were available (N = 45) had stable levels of transgene sequences

(peripheral blood [PB] VCN) detectable through last follow-up. PB VCN in Group C subjects was generally higher than that in Group A subjects, consistent with manufacturing refinements and higher drug product (DP) VCN in Group C. PB VCN values were stable and durable from approximately 6 months after lovo-cel infusion through Month 24, suggesting the persistence of transduced HSCs and their ongoing differentiation into transgene-containing progeny in peripheral blood. Stable PB %LVV+ Cells and %LVV+ BFU-E values over time observed in exploratory assays in Group C support this conclusion.

- The PB VCN observed at any time in a given subject was generally lower than their DP VCN, and this trend was particularly pronounced in subjects in Group A. This most likely reflects different distributions of transduced cells in short-term progenitors versus long-term repopulating HSCs in lovo-cel. Manufacturing refinements implemented prior to Group C lovo-cel manufacture resulted in PB VCN values that were closer to DP VCN values.

All Subjects Produce Transgenic β^{A-T87Q} -globin

- A positive correlation was observed between PB VCN levels and production of HbA^{T87Q}.
- All subjects treated with lovo-cel produced β^{A-T87Q} -globin through last follow-up, with HbA^{T87Q} expression levels generally stabilizing by approximately 6 months after lovo-cel infusion, with median (min, max) values of 5.19 (2.6, 8.8) g/dL in Group C. These levels were maintained through Month 24, with median (min, max) of 5.57 (2.4, 9.4) g/dL. These results indicate sustained expression of the transgene in the progeny of transduced HSCs. Subjects in Group C generally produced higher levels of HbA^{T87Q} than those in Group A, as anticipated due to manufacturing refinements.
- Exploratory assays suggest that the majority of peripheral red blood cells (RBCs) in Group C subjects contained β^{A-T87Q} -globin, and had a decreased amount of sickling under hypoxic conditions compared with RBCs from untreated patients with SCD.

HbS Production Decreased in all Subjects

- HbS and HbA^{T87Q} production showed similar kinetics during hematopoietic reconstitution, with stabilization by Month 6 (median [min, max] of 5.79 [1.6, 7.3] g/dL) and maintenance through Month 24 (median [min, max] 6.04 [3.7, 7.8] g/dL). The amount of HbS was expected to be decreased in the presence of HbA^{T87Q} production. Group C subjects generally had lower median HbS levels than Group A subjects, consistent with higher median HbA^{T87Q} levels in Group C.

α -globin Genotype Influenced Hb Production

- A decreased number of functional α -globin genes was associated with lower total Hb and lower MCH after lovo-cel treatment.

Conclusions (PD-related):

SCD is a progressive disease, characterized by sporadic episodes of intolerable pain, progressive debilitation, and premature death. In many patients with SCD, the disease is relentless. Existing therapies alleviate the burden of disease for some, but leave a substantial unmet medical need in many. Treatment that successfully prevents the RBC sickling that is the pathophysiologic underpinning of ischemic injury would not only provide freedom from painful crises, but would offer the potential to arrest progressive organ system damage and arthropathy.

Results presented in this interim report demonstrate significant clinical benefit from lovo-cel treatment, most notably complete resolution of VOs and sVOEs (90.3% and 96.8%, respectively, for TPVOE Group C) and improved hematologic parameters, with 85.7% of Group C subjects achieving the composite endpoint of Globin Response that evaluates production of HbA^{T87Q} and total non-transfused Hb. Additional assessments provide evidence for reduced hemolysis and stress erythropoiesis, reduced pRBC transfusions, reduced pain, reduced VOE-related hospitalization, and improved quality of life.

The treatment regimen had a safety profile largely reflective of the known effects of myeloablation with busulfan, HSC transplant, and underlying SCD. Lovo-cel was generally well-tolerated, with no AEs attributable to the use of an LVV for the ex vivo transduction of autologous cells (e.g., no detection of vector-derived RCL or insertional oncogenesis). However, AEs related to SCD were common, and included AEs reflecting chronic complications of SCD after treatment.

Overall, results are consistent with the mechanism of action of lovo-cel: the prevention of RBC sickling alleviated acute crises and reduced ischemic consequences, which are anticipated to diminish the morbidity and mortality associated with SCD. Gene therapy is not anticipated to reverse established body and organ system pathology, and thus treated subjects may experience an ongoing burden of disease resulting from the chronic effects of SCD tissue injury.

Source: Applicant. Module 5, section 5.3 Clinical Study Reports.

7.3 Study #3 – Study HGB-210

Interim Analysis Data Cutoff Date: August 01, 2022

<p>Title: A Phase 3 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the BB305 Lentiviral Vector in Subjects with Sickle Cell Disease.</p>
<p>Objectives: Primary: Evaluate the efficacy of treatment with lovo-cel in subjects with SCD. Secondary: Evaluate the safety of treatment with lovo-cel in subjects with SCD.</p>
<p>Methodology: This is a non-randomized, open-label, multi-site, single-dose, Phase 3 study in approximately 35 adults and pediatric subjects ≥ 2 and ≤ 50 years of age with SCD. Informed consent for pediatric subjects proceeded in a staggered fashion with respect to age. After at least 2 subjects ≥ 12 and < 18 years of age attained neutrophil engraftment (NE) after drug product infusion in SCD studies, the Data Monitoring Committee (DMC) reviewed their safety data and recommended that Study HGB-210 could safely proceed with treatment of subjects ≥ 5 and < 12 years of age. Treatment is divided into 4 stages, as follows: Stage 1: screening and eligibility assessment; Stage 2: stem cell harvest, drug product manufacture and disposition; Stage 3: myeloablative conditioning and infusion of lovo-cel; Stage 4: Follow-up, through 24 months after drug product infusion.</p>
<p>Number of Subjects: Approximately 35 subjects were planned to be treated. At the interim data cut-off for this interim analysis, 2 subjects had been treated with lovo-cel (and enrollment is ongoing).</p>
<p>Main Criteria for Inclusion/Exclusion: Key common inclusion/exclusion criteria for all subjects: Subjects must be: ≥ 2 and ≤ 50 years of age at time of consent (or assent, as applicable), with SCD (diagnosis with either β^S/β^S, β^S/β^0, or β^S/β^+ genotype); and a willing, matched human leukocyte antigen (HLA) identical sibling hematopoietic cell donor is not available. Subjects with genetic mutations that result in the inactivation of 2 or more α-globin genes were excluded from enrollment. Subjects must have at least 4 protocol-defined vaso-occlusive events (VOEs) prior to informed consent, adequate bone marrow function, no contraindications to plerixafor during the mobilization of HSCs or use of busulfan or other medicinal products required during myeloablative conditioning. Subjects must have no history of stroke or stage IV neurovasculopathy, no history of iron overload or Cardiac T2* magnetic resonance imaging (MRI) < 10 msec, no contraindication to anesthesia, and no findings of advanced liver disease. Subjects must have either experienced hydroxyurea (HU) failure at any point in the past (defined as > 1 VOE or ≥ 1 acute chest syndrome (ACS) after HU has been prescribed for at least 6 months) or must have intolerance to HU (intolerance is defined as the patient being unable to continue to take HU per Principal Investigator (PI) judgment). Subjects must not have presence of a chromosomal abnormality or genetic mutation that may put them at an increased risk of myelodysplastic syndrome or acute myeloid leukemia (AML) per Investigator's judgment. In cases when a chromosomal abnormality or genetic mutation is present but not determined to be exclusionary by the Investigator, approval of the Sponsor's Medical Monitor will be required.</p>

before the subject is considered eligible for the study.

Study Treatments

Investigational product(s): LentiGlobin BB305, lovo-cel

lovotibeglogene autotemcel suspension for infusion (also known as bb1111 or LentiGlobin BB305 Drug Product for Sickle Cell Disease (SCD); hereafter referred to as lovo-cel)

Lovo-cel is an autologous CD34+ cell-enriched population from patients with SCD that contains HSC transduced with BB305 LVV encoding the β^{A-T87Q} -globin gene, suspended in a cryopreservation solution. Each drug product was prepared individually for each subject using his/her autologous cells.

Subjects were to receive lovo-cel at a dose of $\geq 3.0 \times 10^6$ CD34+ cells/kg, administered as a single IV dose on a single day. More than 1 drug product lot may have been required to reach the minimum cell dose. The lovo-cel lot numbers for treated subjects in this interim CSR were: BB03-20-013 and BB03-20-019.

Pharmacodynamic Results:

- Both treated subjects for whom data are available (N = 2) have detectable transgene sequences in the peripheral blood vector copy number (PB VCN) through last follow-up, with PB VCN levels of 3.4273 and 3.0901 copies per diploid genome (c/dg) at Month 6. These parameters were stable and durable from approximately 6 months after drug product infusion through longest time observed (15 months after drug product infusion).
- Both subjects treated with lovo-cel produced β^{A-T87Q} -globin through last follow-up, with HbA^{T87Q} levels generally stabilizing by approximately 6 months after drug product infusion, producing 5.62 g/dL and 7.53 g/dL HbA^{T87Q} at Month 6. These parameters were stable and durable from approximately 6 months after drug product infusion through longest time observed (15 months after drug product infusion).
- HbS and Total non-transfused Hb over time increase initially as the subject's hematopoietic system is reconstituted, with stabilization of levels approximately 6 months after lovo-cel infusion. Total non-transfused Hb levels reached 11.01 g/dL and 14.11 g/dL, and HbS levels reached 4.66 g/dL and 5.68 g/dL at Month 6 and then remained relatively stable.
- The α -globin/ β -like globin ratios were stable at approximately 1.1 from Month 6 through last visit

Conclusions:

Both subjects treated with lovo-cel experienced clinical benefit from treatment, with complete resolution of all VOs and sVOs, and achievement of the composite endpoint of GR. Safety data to-date demonstrate that the treatment regimen including cell procurement, conditioning, and autologous transplant has been generally well tolerated. At the time of this interim CSR, data from Study HGB-210 are limited but support continuation of the investigation of lovo-cel in patients with SCD.

Source: Applicant. Module 5, section 5.3 Clinical Study Reports.

7.4 Study #4 – Study LTF-307

Interim Analysis Data Cutoff Date: August 18, 2022

Title: Long-term Follow-up of Subjects With Sickle Cell Disease Treated With Ex Vivo Gene Therapy Using Autologous Hematopoietic Stem Cells Transduced With a Lentiviral Vector

Objectives:

- Evaluate long-term safety of treatment with lovo-cel in subjects with SCD
- Evaluate long-term efficacy of treatment with lovo-cel in subjects with SCD

Methodology:

This study is a multi-center, long-term safety and efficacy follow-up study for subjects with sickle cell disease (SCD) who have been treated with lovo-cel in the Sponsor's parent studies HGB-205 and HGB-206. After monitoring of a subject in the parent study was complete (where the parent study includes approximately 2 years of follow-up after drug product infusion), subjects were eligible to enroll in a long-term follow-up study.

Twenty-one subjects were initially enrolled in Study LTF-303, a long-term follow-up study for subjects who received treatment either for β -thalassemia or SCD. Subjects treated with lovo-cel were subsequently enrolled in SCD-specific Study LTF-307, as applicable, and their data transferred to the LTF-307 database. No subjects from Phase 3 Study HGB-210 had completed the 2-year parent study as of this interim data cut and therefore none were enrolled in long-term follow-up. During Study LTF-307, subjects are planned to be monitored every 6 months through Month 60, and yearly thereafter through 15 years post-drug product infusion.

Number of Subjects:

Results are presented for the enrolled subjects from Studies HGB-205 (N = 3) and HGB-206 (N = 38) as of the data cut-off date for this interim LTF-307 CSR (18 August 2022). The number of subjects planned for enrollment in Study LTF-307 is dependent on the number of subjects completing each parent study who meet all eligibility criteria and provide written informed consent, or assent as applicable, for participation in Study LTF-307.

Main Criteria for Inclusion:

Treatment with lovo-cel in a bluebird bio-sponsored clinical study.

Study Treatments

No investigational treatment was administered in this study.

Clinical Pharmacology Results:*All Subjects Maintain Integrated Transgene in Peripheral Blood Leukocytes*

- All subjects evaluated (N = 41) had detectable transgene sequences (peripheral blood [PB] VCN) in the peripheral blood through last follow-up or last follow-up prior to malignancy diagnosis if subject had malignancy. These parameters were stable and durable during this long-term follow-up study, with longest follow-up of approximately 7 years after drug product infusion. Results demonstrate persistence of transduced hematopoietic stem cells (HSCs) and their ongoing differentiation into transgene-containing progeny in peripheral blood.
- Exploratory assays on PB percentage of cells with transgene sequences (%LVV+) Cells and %LVV+ burst forming unit erythroids over time have limited data but additionally support stable and durable persistence of transduced HSCs.

All Subjects Produce Transgenic β^{A-T87Q} -globin

- All subjects produced β^{A-T87Q} -globin through last follow-up or last follow-up prior to malignancy diagnosis if subject had malignancy, with HbA^{T87Q} levels stable and durable, with longest follow-up at approximately 7 years after drug product infusion. These results indicate sustained expression of the transgene in the progeny of transduced HSCs.
- Exploratory analyses with limited data suggest that other pharmacodynamic parameters dependent on HbA^{T87Q} production, including % β^{A-T87Q} -globin positive red blood cells (RBCs) and extent of sickling under hypoxic conditions, are also stably maintained through to last evaluation in this long-term follow-up study.

Conclusions:

SCD is a progressive disease in which patients cope with increased disease burden over time, and development of severe disease is inevitable. VOs and ensuing damage start in infancy, with the frequency and intensity of VOs increasing in the adolescent population and peaking in the adult population. VOs are correlated with an increased risk of sudden death and cumulative progressive organ damage. Regardless of available treatments, SCD patients face increased risk of early death, with anticipated life expectancy estimated to be 20 to 30 years lower than that for unaffected individuals. Prognosis has not substantially improved in the United States in the

past 2 decades.

Results presented in this interim report on long-term outcomes demonstrate significant and durable clinical benefit from lovo-cel treatment, most notably maintained resolution of VOEs and sVOEs for most subjects, maintenance of improved hematologic parameters, reduced hemolysis markers, reduced pRBC transfusions, reduced pain, and reduced VOE-related hospitalization. Improvements in quality of life were generally maintained for adult subjects, including improvements in pain, pain interference, and fatigue.

The safety profile observed during long-term follow-up primarily reflects acute and chronic complications of underlying SCD. Most of these events occurred in subjects treated with a drug product manufactured prior to the refined process. Two subjects treated in HGB-206 Group A developed malignancy and died, and although neither case represented insertional oncogenesis, hematologic malignancy is considered an important identified risk of lovo-cel.

Overall, long-term results are consistent with the mechanism of action of lovo-cel: the prevention of RBC sickling alleviated acute crises, and the reduction of widespread ischemic consequences is anticipated to diminish the morbidity and mortality associated with SCD. Gene therapy is not anticipated to reverse established bony and organ system pathology, and thus treated subjects may experience an ongoing burden of disease resulting from the chronic effects of SCD tissue injury.

Interim results presented here demonstrate durability of lovo-cel over time and therefore support the case for availability of lovo-cel treatment to SCD patients, especially in light of the ongoing health burden, progressive and worsening tissue injury, and reduced life expectancy among SCD patients in the United States.

Source: Applicant. Module 5, section 5.3 Clinical Study Reports.

7.5 Study #5 – Population Pharmacodynamic Analysis (Study # 271953)

The Applicant developed population pharmacodynamic (PD) model to 1) describe the PB VCN profile following an IV infusion of LYFGENIA in patients with SCD; 2) describe the disposition of HbA^{T87Q} concentrations following an IV infusion of LYFGENIA in patients with SCD; and 3) derive individual model predicted values of steady-state PB VCN and HbA^{T87Q}, and to determine the time to reach 90% to 95% of the steady-state PB VCN and HbA^{T87Q} values.

(b) (4) were used in PopPD analysis.

7.5.1 Data Source

Population PD models were built using data from Studies HGB-205, HGB-206, HGB-210, and the long-term follow-up study LFT-307 (Table 10). The model building of (b) (4) dataset consisted of 772 samples from 35 subjects, with a total of 385 PB VCN and 387 HbA^{T87Q} concentrations.

Table 10. Overview of Clinical Studies

Study	Study Description	Subjects (N) [†]	Process	Sampling Schedule [‡]
HGB-205	Phase 1/2 open label study evaluating the safety and efficacy of bb1111 in SCD	3	0	Months 1, 2, 3, 4.5, 6, 9, 12, 15, 18, 21, 24
HGB-206	Phase 1/2 study evaluating bb1111 in subjects with severe sickle cell disease	Group A: 7	Group A: 1	Months 1, 2, 3, 4.5, 6, 9, 12, 15, 18, 21, 24
		Group B: 2	Group B: 1,2	

		Group C: 36	Group C: 2a	
HGB-210	Phase 3 study evaluating bb1111 in subjects with severe sickle cell disease	2	2a	Months 1, 2, 3, 4.5, 6, 9, 12, 15, 18, 21, 24
LTF-307	Long-term follow-up of subjects with sickle cell disease treated with bb1111	41	Not applicable*	Months 30, 36, 42, 48, 54, 60 Yearly from 6-15 years post-drug product infusion

*Number of subjects with SCD infused with bb1111.

*Sampling times for PB VCN and HbA^{T87Q}.

*No subjects were treated during this long-term follow-up.

Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.

Four manufacturing processes were used in the development of LYFGENIA (Table 11).

Table 11. Manufacturing Process Attributes

Process	bb1111 LVV	Source	Study/Group
0	aLVV	Bone Marrow	HGB-205
1	aLVV	Bone Marrow	HGB-206, Group A and B
2	aLVV	Bone Marrow	HGB-206, Group B
2a	aLVV and sLVV	Apheresis	HGB-206, Group C, HGB-210

aLVV = adherent culture LVV, sLVV = suspension culture LVV.

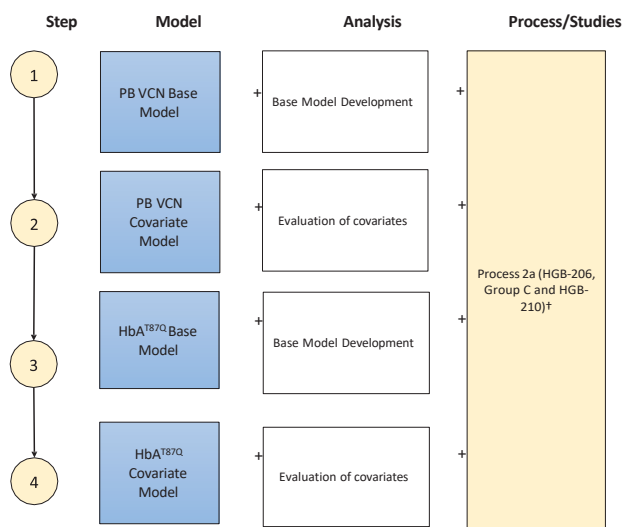
Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.

There were 3 subjects who received these mixed LVV type drug products. The 3 subjects were included in all pooled analyses; however, they were excluded from the analyses performed to investigate differences in outcome based on LVV type.

7.5.2 Methods

The population PB VCN and HbA^{T87Q} models were developed following the steps outlined in below Figure.

Figure 14. PopPD Model Development Steps



Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.

The covariates that were considered in the analysis are provided in the table below.

Table 12. Covariates evaluated in popPD model development

Covariate	Code	Value
Age at baseline (y)	AGE	Continuous
Age at baseline (y)	AGECAT	Categorical
Weight at baseline (kg)	WT	Continuous
Weight at baseline (kg)	WTCAT	Categorical
Sex	SEX	Categorical
DP VCN	DPVCN	Continuous
Weighted Average of DP VCN/DP %LVV + Cells	DPVCNLVV	Continuous
Manufacturing Process	MANF	Categorical
LVV type	LVVT	Categorical
Genotype [†]	GENTYP	Categorical
DP %LVV+ Cells	DPLVVP	Continuous
Average busulfan AUC	BUSAUCA	Continuous
PB VCN Model Parameters [‡]		Continuous

[†]Alpha-globin genotypes aa/aa, aa/-a3.7 and -a3.7/-a3.7 were assessed. [‡]Parameters from the PBVCN model such as steady-state PB VCN (VCN_{MAX}) were tested as covariates in the HbA^{T87Q} model.

Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.

7.5.3 Results

7.5.3.1 Population PD Model for PB VCN

The PB VCN PD profile was best described by an asymptotic growth model with empiric time based function with below equation. PB VCN was modeled in terms of steady-state PB VCN (VCN_{MAX}), rate of transgene appearance (KTA) and an empirical time-based function to account for the different time course shapes. Between-subject variability (BSV) was incorporated on the

VCNMAX, KTA, and shape (γ) parameters. A proportional error model was used to describe the residual unexplained variability (RUV).

(b) (4)

Here, VCNMAX = steady-state PB VCN, FC = fold change in PB VCN, t = time, γ = shape parameter, and KTA = rate of transgene appearance.

As shown in Table 13, there were two significant covariates in the final model: LVV type on VCN_{MAX} and fold change in PB VCN (FC) and age on FC (Figure 15).

Table 13. Parameter Estimates for the final PB VCN Model

Parameter Name	Estimated Value (%RSE)	Bootstrap Median (95% CI) ^a
Estimate of VCN _{MAX} (c/dg)	1.27 (8.7)	1.27 (1.07 – 1.52)
Fold increase in VCN _{MAX} for sLVV (unitless)	1.7 (20.5)	1.7 (1.3 – 2.3)
Estimate of K _{TA} (/month)	2.01 (15.1)	1.93 (1.35 – 2.87)
Estimate for fold change (FC) in VCN _{MAX} (unitless)	1.59 (11.5)	1.63 (1.30 – 2.31)
Fractional change in FC for sLVV (unitless)	-0.633 (15.0)	-0.634 (-0.801 – -0.400)
Exponent for age covariate on FC (unitless)	0.959 (24.0)	0.984 (0.509 – 1.52)
Between Subject Variability for VCN _{MAX} (%CV)	43.8 (12.6)	42.3 (31.8 – 53.4)
Between Subject Variability for K _{TA} (%CV)	31.6 (31.2)	29.4 (31.6 – 61.4)
Between Subject Variability for FC (%CV)	36.8 (16.6)	33.9 (36.8 – 47.3)
Residual Unexplained Variability (Proportional, %CV)	8.81 (5.1)	8.72 (7.86 – 9.58)

%RSE = percent relative standard error. %CV = approximate coefficient of variation. ^a95% CI = confidence interval is reported from the 2.5th and 97.5th percentiles of the estimated values from 998 bootstrap datasets.

Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.

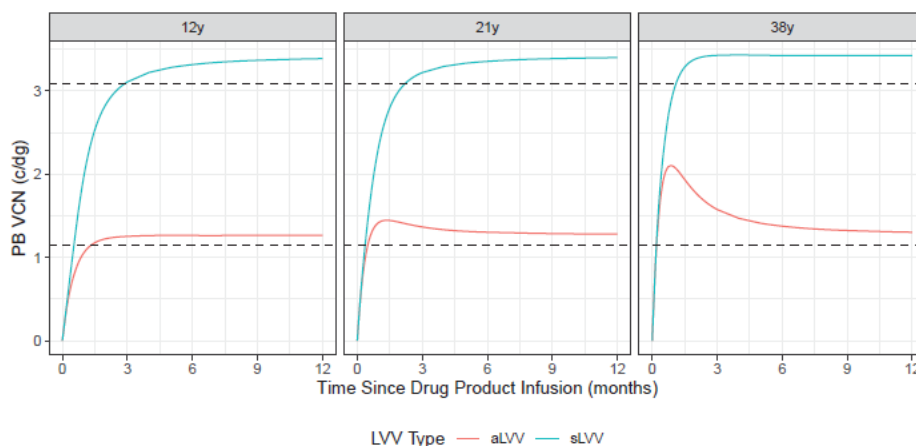
LVV type on VCN_{MAX} and fold of change in PB VCN (FC):

- DP manufactured with suspension culture LVV (sLVV) results in a (b) (4)-fold higher steady-state PB VCN (95% prediction interval (PI) 2.01 – 3.55) compared to DP manufactured with adherent culture LVV (aLVV). Due to the very limited number of subjects received sLVV LYFGENIA (n=6), the results should be interpreted with caution.
- DP manufactured with sLVV was predicted to have a longer time to 90% steady state PB VCN of 2.8, 2.2, and 1.1 months compared to 1.3, 0.5, and 0.2 months for those that received DP manufactured with aLVV for subjects aged 12, 21, and 38 years, respectively.

Age on fold of change in PB VCN (FC):

Age was predicted to have a minor impact on time to 90% steady-state PB VCN, with ≤ 1.7 months difference predicted between the youngest (12 years) and oldest age (38 years) of subjects recruited in studies HGB-206 Group C and HGB-210 that received DP Process 2a.

Figure 15. Effect of LVV Type and Age on PB VCN PD Profile



Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.

There was no evidence of a statistically significant relationship between PB VCN and the following covariates; percent lentiviral vector positive cells in Drug Product (DP %LVV+ Cells), Drug Product vector copy number (DP VCN), genotype, sex, weight, and average busulfan area under the plasma concentration-time curve (AUC) (all p values >0.005).

7.5.3.2 Population PD Model for HbA^{T87Q}

The HbA^{T87Q} PD profile was best described by a sigmoidal maximum effect (E_{max}) model. HbA^{T87Q} was modeled in terms of steady-state HbA^{T87Q} (HbA^{T87Q}MAX), time to 50% maximal HbA^{T87Q} (ET₅₀, HbA^{T87Q}), and a Hill slope (γ). BSV was incorporated on all three structural parameters. A proportional error model was used to describe the RUV. The form of the sigmoidal E_{max} is shown in following equation:

$$(b) \quad (4)$$

Here, VCNMAX = steady-state PB VCN, FC = fold change in PB VCN, t = time, γ = shape parameter, and KTA = rate of transgene appearance.

Two statistically significant covariates were identified in the final model individual VCN_{MAX} on steady-state HbAT87Q (HbA^{T87Q}_{MAX}) and genotype on time to 50% maximal HbA^{T87Q} (ET₅₀, HbAT87Q).

Table 14. Parameter Estimates for the final HbA^{T87Q} Model

Parameter Name	Estimated Value (%RSE)	Bootstrap Median (95% CI)
Estimate of HB _{MAX} (c/dg)	6.02 (10.2)	5.92 (5.31 – 7.57)
E _{max} for VCN covariate	1 FIX	1 FIX
Estimate VCN _{MAX,50} for sLVV (c/dg)	0.442 (19.1)	0.478 (0.340 – 0.679)
Hill Slope of VCN covariate (γ_{VCN} , unitless)	1.81 (27.5)	1.99 (1.08 – 4.23)
Estimate of E _{50,pop} (/month)	2.04 (3.4)	2.04 (1.92 – 2.18)

Change in E ₅₀ for -A3.7/-A3.7 genotype (fraction)	0.803 (16.0)	0.801 (0.579 – 1.07)
Hill slope (γ, unitless)	3.19 (4.0)	3.20 (2.96 – 3.46)
Between Subject Variability for HB _{MAX} (%CV)	18.8 (25.0)	17.8 (9.9 – 24.1)
Between Subject Variability for ET _{50, HbA^{T87Q}} (%CV)	18.4 (13.6)	18.0 (12.9 – 22.6)
Between Subject Variability for Hill Slope (%CV)	20.7 (13.1)	20.3 (12.9 – 22.6)
Residual Unexplained Variability (Proportional, %CV)	12.3 (17.9)	12.3 (8.1 – 16.7)

%RSE = percent relative standard error. %CV = approximate coefficient of variation. 95% CI = confidence interval is reported from the 2.5th and 97.5th percentiles of the estimated values from 984 bootstrap datasets

Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.

Individual VCN_{MAX} (from the PB VCN model) on steady-state HbA^{T87Q} (HbA^{T87Q}_{MAX})

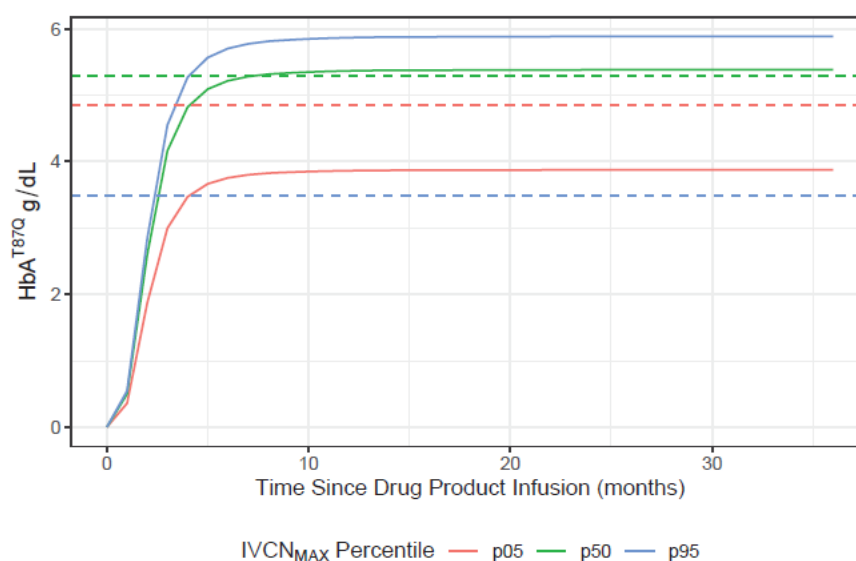
- Subjects with the 5th percentile of VCN_{MAX} (0.614 c/dg) were predicted to have a 0.71-fold change (95% PI 0.5 – 1.01) in steady-state HbA^{T87Q} relative to the median VCN_{MAX} (1.448 c/dg).
- Subjects with the 95th percentile of VCN_{MAX} (3.663 c/dg) were predicted to have a 1.1-fold change (95% PI 0.86 – 1.35) in steady-state HbA^{T87Q} relative to the median VCN_{MAX} (1.448 c/dg).

Genotype on time to 50% maximal HbA^{T87Q} (ET_{50, HbA^{T87Q}})

- Subjects with the -A3.7/-A3.7 genotype were predicted to have a 1.91-fold longer time to 90% steady-state HbA^{T87Q} (95% PI 1.5 – 2.35) compared to subjects with either the AA/AA or AA/-A3.7 genotypes, however this result should be interpreted with caution as there were only two subjects with the -A3.7/-A3.7 genotype in the analysis.

There was no evidence of a statistically significant relationship between HbA^{T87Q} and the following covariates: DP %LVV+ Cells, DP VCN, age, sex, weight, and average busulfan AUC (all p values >0.005).

Figure 16. Effect of Individual VCN_{MAX} on HbA^{T87Q} PD Profile

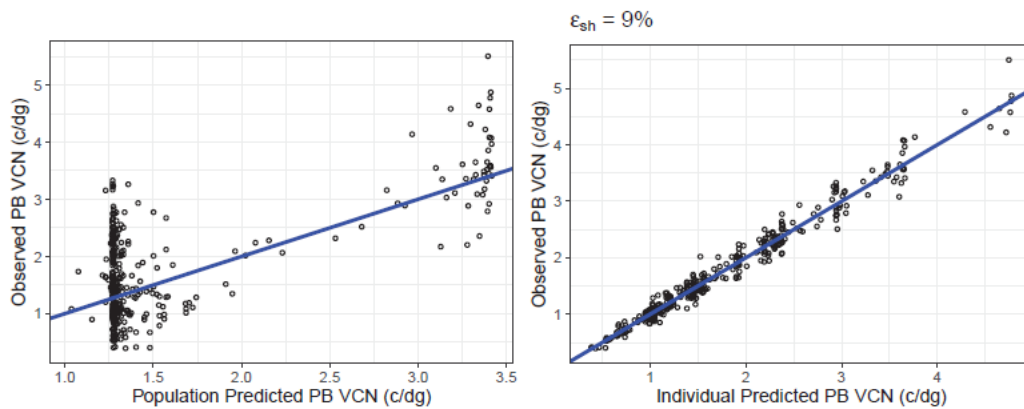


Solid lines = HbA^{T87Q} profile. Dashed lines = 90% value of maximum HbA^{T87Q}

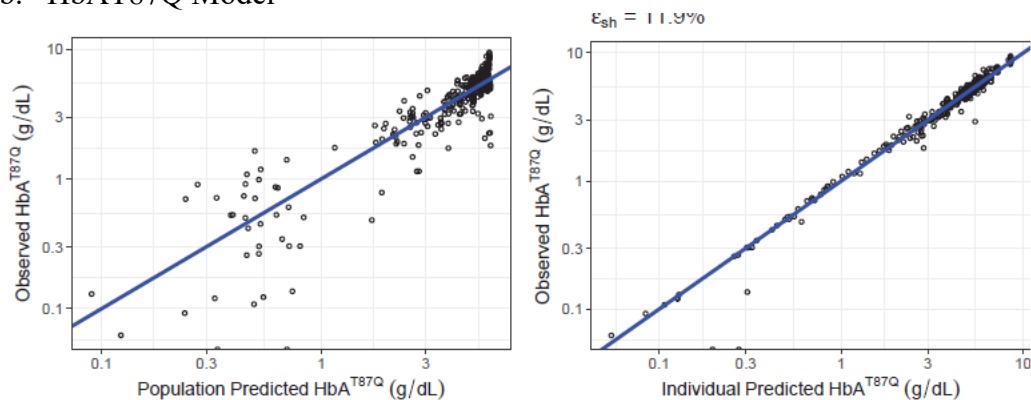
Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.

Figure 17. Goodness of Fit Plots for Final PopPD Models

a. PB VCN Model



b. HbAT87Q Model



The blue lines represent the line of unity, and ϵ_{sh} shows shrinkage for the residual variability. Note: x and y-axis are on log scale.

Source: Applicant. Module 5, section 5.3.5.3. Population Pharmacodynamic Analysis Report.