

Clinical Pharmacology BLA Review
Division of Clinical Evaluation and Pharmacology/Toxicology
Office of Tissues and Advanced Therapy

BLA	125717/0
Product	Zynteglo (betibeglogene autotemcel, beti-cel) Suspension for Intravenous Infusion, (b) (4) – 2.0 x 10 ⁶ cells/mL
Sponsor	Bluebird Bio, Inc.
Indication	Treatment of patients with β-thalassemia who require regular red blood cell (RBC) transfusions
Date Received	September 20, 2021
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1 EXECUTIVE SUMMARY

Bluebird Bio, Inc. seeks approval of its BLA for ZYNTÉGLO (betibeglogene autotemcel, beti-cel, LentiGlobin BB305) for the treatment of patients with β -thalassemia who require regular red blood cell (RBC) transfusions. ZYNTÉGLO is a gene therapy consisting of autologous CD34+ cells containing hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding β^{A-T87Q} -globin. ZYNTÉGLO is a cell suspension for a one time single dose intravenous infusion. The proposed minimum dose of ZYNTÉGLO is 5.0×10^6 CD34+ cells/kg.

The clinical pharmacology evaluation of this biologics license application (BLA) is based on data from four clinical studies (one Phase 1/2, two Phase 3, and a long-term follow up study) and a population pharmacodynamic study. After infusion of ZYNTÉGLO, transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce red blood cells (RBCs) containing biologically active β^{A-T87Q} -globin that will combine with α -globin to produce functional Hb containing β^{A-T87Q} -globin (HbA^{T87Q}). HbA^{T87Q} generally increased steadily after administration of ZYNTÉGLO, and stabilized by approximately Month 6 post-infusion. In the ongoing Phase 3 studies (Studies HGB-207 and HGB-212), subjects with transfusion-dependent β -thalassemia (TDT) had a Month 6 median (min, max) HbA^{T87Q} of 8.74 (0.00, 12.01) g/dL (N = 35). HbA^{T87Q} remained durable with a median (min, max) of 8.80 (0.34, 12.43) g/dL at Month 24 (N = 30), demonstrating sustained expression of the β^{A-T87Q} protein derived from irreversible integration of the β^{A-T87Q} -globin gene into long-term HSCs. The efficacy of ZYNTÉGLO was established based on achievement of transfusion independence (TI), which is defined as a weighted average Hb ≥ 9 g/dL without any pRBC transfusions for a continuous period of ≥ 12 months at any time during the study, after infusion of ZYNTÉGLO. Among 36 evaluable subjects in Phase 3 studies, 32 (88.9%, 95% CI: 73.9, 96.9) achieved TI.

The proposed dosing regimen of ZYNTÉGLO administered by intravenous (IV) infusion 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

ZYNTÉGLO (betibeglogene autotemcel, beti-cel, LentiGlobin BB305) is a gene therapy designed to provide a functional β -globin gene to patients with TDT, thus eliminating their need for chronic RBC transfusions. ZYNTÉGLO (beti-cel) is an autologous CD34+ cell-enriched population that contains HSCs transduced with lentiviral vector (LVV) encoding β^{A-T87Q} -globin gene, suspended in cryopreservation solution containing 5% dimethyl sulfoxide (DMSO). CD34+ cells contain long-term HSCs which are necessary for the sustained, permanent production of β -globin-expressing cells during erythropoiesis. β^{A-T87Q} -globin is identical to human β -globin except for a single amino acid change from threonine (T) to glutamine (Q) at position 87, with the new amino acid at that position also being found in normal delta- and gamma-globin chains. The single acid change conserves the protein's function while allowing for quantification relative to other globin species.

β -Thalassemia is caused by the absence or reduced production of the β -globin chains of hemoglobin A (HbA), resulting in an excess of uncomplexed α -globin proteins that precipitate in the erythroblasts leading to premature death of the cells, ineffective erythropoiesis, and hemolysis. ZYNTÉGLO contains functional copies of a modified β -globin gene in patient HSCs introduced of autologous CD34+ cells with LVV encoding β^{A-T87Q} -globin gene. After intravenous infusion of ZYNTÉGLO, transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce red blood cells (RBCs) containing biologically active β^{A-T87Q} -globin (a modified β -globin protein) that will combine with α -globin to produce functional Hb containing β^{A-T87Q} -globin (HbA^{T87Q}).

ZYNTÉGLO is a cell suspension for a single dose (one time treatment) intravenous infusion and the proposed minimum dose of ZYNTÉGLO is 5.0×10^6 CD34+ cells/kg.

This clinical pharmacology section of this application is supported by two Phase 1/2 studies (Study # HGB-205, HGB-204), two Phase 3 studies (Studies HGB-207, HGB-212) and one long-term follow up study (Study # LTF-303). The Applicant also submitted a population pharmacodynamic study. Due to lack of drug product comparability information, Study #HGB-205 is generally excluded from analysis in current review.

3 SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY FINDINGS

ZYNTÉGLO (beti-cel) is a gene therapy consisting of autologous CD34+ cells containing hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding β^{A-T87Q} -globin. After infusion of ZYNTÉGLO, transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce red blood cells (RBCs) containing biologically active β^{A-T87Q} -globin that will combine with α -globin to produce functional Hb containing β^{A-T87Q} -globin (HbA^{T87Q}).

General Pharmacodynamics

- After infusion of ZYNTÉGLO, 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 levels in the Phase 3 studies was 1.293 (0.16, 4.52) c/dg (N=37). PB VCN levels generally remained stable as of the data cut off data of all studies. High inter-subject variability of PB VCN kinetic profiles was observed.
- HbA^{T87Q} generally increased steadily after administration of ZYNTÉGLO, and stabilized by approximately Month 6 post-infusion. In the ongoing Phase 3 studies (Studies HGB-207 and HGB-212), subjects with TDT had a Month 6 median (min, max) HbA^{T87Q} of 8.74 (0.00, 12.01) g/dL (N = 35). HbA^{T87Q} remained durable with a median (min, max) of 8.80 (0.34, 12.43) g/dL at Month 24 (N = 30), demonstrating sustained expression of the β^{A-T87Q} protein derived from irreversible integration of the β^{A-T87Q} -globin gene into long-term HSCs.
- Intrinsic factors, such as genotype, age at baseline, race, sex, and weight at baseline did not impact the steady-state levels and time to steady-state for PB VCN and HbA^{T87Q}.
- Analysis of Hemoglobin (Hb) showed that HbA^{T87Q} was the major contributor to unsupported total Hb. The relative contributions of endogenous Hb may differ for each individual subject.
- At Month 6 post-infusion of ZYNTÉGLO, the median (min, max) unsupported total Hb levels were 11.55 (8.4, 13.3) g/dL (N=22) and 10.20 (8.5, 13.2) g/dL (N=15) in Phase 3 Studies HGB-207 and HGB-212, respectively. The median unsupported total Hb levels were > 10 g/dL during the observation period of Phase 3 studies.

Dosing Characteristics and Responses

- 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 plateaued at higher levels of DP VCN after reaching approximately 80% to 90% DP %LVV+ cells. Positive correlation was also observed between DP %LVV+ cells and PB VCN levels at Month 6 post ZYNTÉGLO infusion. Population PD analysis

also indicated that DP %LVV+ cells was the most important covariate impacting PB VCN levels.

- There was a significant correlative relationship between DP VCN and HbA^{T87Q} levels at Month 6 after infusion of ZYNTGLO. HbA^{T87Q} increased gradually with DP VCN at lower DP VCN values, but at higher DP VCN values, HbA^{T87Q} levels plateaued at approximately 8 to 10 g/dL. This observation indicates a feedback regulation of β -globin (or total Hb) levels within erythroid cells to maintain β -globin (or total Hb) levels below certain upper levels.
- There was a significant correlative linear relationship between DP %LVV+ cells and HbA^{T87Q} at Month 6. Subjects received ZYNTGLO with higher DP %LVV+ cells had higher HbA^{T87Q} at Month 6, compared to subjects received ZYNTGLO with lower DP %LVV+ cells.
- No correlation between ZYNTGLO subpopulations and PB VCN and HbA^{T87Q} at post-infusion Month 6 and 24 were observed.
- There was no correlation observed between total CD34+ cell dose and HbA^{T87Q} in peripheral blood at either Month 6 or Month 24 for the *All TDT* pool, indicating that the lowest cell dose evaluated to date was adequate for effective reconstitution of HSCs in treated subjects.
- The targeted AUC range of busulfan evaluated in clinical studies was considered adequate for myeloablation.

Pharmacodynamic Responses and Transfusion Independence (TI)

- PB VCN and HbA^{T87Q}: HbA^{T87Q} increased quickly with the increase of PB VCN at lower PB VCN levels, followed by an HbA^{T87Q} plateau at higher PB VCN levels. This observation suggests the regulation of β -globin levels within erythroid cells and selection of erythroid cells producing β^{A-T87Q} -globin during engraftment for balanced α -globin/ β -globin ratios.
- HbA^{T87Q} and transfusion independence (TI): Subjects with higher HbA^{T87Q} levels were less likely to need blood transfusion. The median (min, max) HbA^{T87Q} at the time when no blood transfusion was needed was 8.44 (0.75, 13.85) g/dL and the median (min, max) HbA^{T87Q} at the time that subjects had blood transfusion was 0.88 (0.00, 5.06) g/dL.
- Unsupported total Hb at Month 6 and transfusion independence (TI): All of the 31 subjects who had ≥ 9 g/dL unsupported total Hb at Month 6 achieved TI. Statistically significant association was observed between unsupported total Hb level (≥ 9 g/dL) at Month 6 and TI.

4 LABELING COMMENTS

The clinical pharmacology reviewer has reviewed the package insert for BLA 125717 and finds it acceptable pending the following revisions shown below.

12. CLINICAL PHARMACOLOGY

12.1. Mechanism of Action

ZYNTEGLO adds functional copies of a modified β -globin gene into patients' HSCs through transduction of autologous CD34+ cells with BB305 LVV, ~~thereby addressing the underlying genetic cause of β -thalassemia.~~ After ZYNTEGLO infusion, transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce RBCs containing biologically active β^{A-T87Q} -globin (a modified β -globin protein) that will combine with α -globin to produce functional adult Hb containing β^{A-T87Q} -globin (HbA^{T87Q}). β^{A-T87Q} -globin can be quantified relative to other globin species in peripheral blood using high performance liquid chromatography. β^{A-T87Q} -globin expression is designed to correct the β/α -globin imbalance in erythroid cells of patients with β -thalassemia and has the potential to increase functional adult HbA and total Hb to normal levels and eliminate dependence on regular pRBC transfusions. ~~Following successful engraftment and achievement of transfusion independence, the effects of ZYNTEGLO are expected to be life-long.~~

12.2. Pharmacodynamics

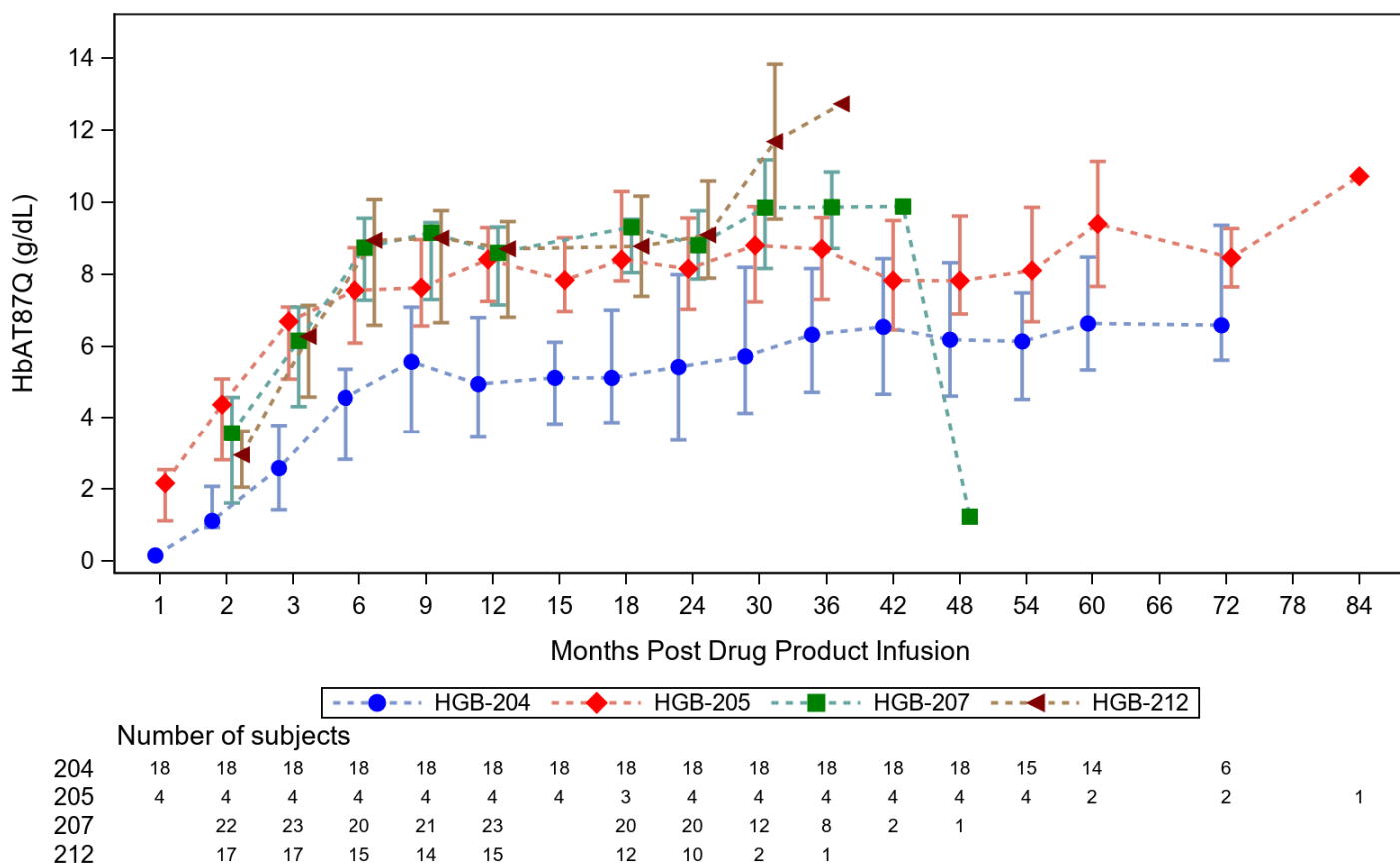
Reviewer's Comments to Applicant:

Please only include Phase 3 data in which the commercial version of the products were used.

HbA^{T87Q} generally increased steadily after ZYNTEGLO infusion and stabilized by approximately Month 6 after infusion (Figure 1). Patients had a Month 6 median (min, max) HbA^{T87Q} of 4.783 (0.43, 9.59) g/dL in the Phase 1/2 studies, HGB-204 and HGB-205 (N = 22) and 8.737 (0.00, 12.01) g/dL in the ongoing Phase 3 studies, HGB-207 and HGB-212 (N = 35).

HbA^{T87Q} remained durable with a median (min, max) at Month 60 of 6.785 (2.84, 11.15) g/dL in the Phase 1/2 studies (N = 16) and 8.802 (0.34, 12.43) g/dL at Month 24 in the ongoing Phase 3 studies (N = 30). HbA^{T87Q} in the Phase 1/2 studies continued to remain durable at last follow-up through Year 7, and through Month 36 in the Phase 3 studies, demonstrating sustained expression of the β^{A-T87Q} protein derived from irreversible integration of the β^{A-T87Q} -globin gene into long-term HSCs.

Figure 1: Median of HbA^{T87Q} Over Time^{a,b}



^a Bars represent interquartile ranges

^b Only one patient in Study HGB-207 had HbA^{T87Q} data at Month 48; this patient did not achieve TI, which accounts for the drop in median HbA^{T87Q} for Study HGB-207 at Month 48

12.3. Pharmacokinetics

ZYNTeglo is an autologous gene therapy ~~which includes consisting of~~ HSCs that have been genetically modified *ex vivo*. The nature of ZYNTeglo is such that conventional studies on pharmacokinetics, absorption, distribution, metabolism, and elimination are not applicable.

5 RECOMMENDATIONS

The clinical pharmacology information in this BLA is acceptable, provided that satisfactory agreement is reached between the sponsor and the FDA regarding the language in Section 12 of the package insert. Please refer to section 4 for detailed Labeling Recommendations.

6 COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

Reviewer's Comments:

As shown in Table 1, clinical development of ZYNTGLO (beti-cel)¹ includes five clinical studies using drug product manufactured from three manufacturing processes: Process 0 (Phase 1/2 Study HGB-205), Process 1 (Phase 1/2 Study HGB-204), and Process 2 (commercial batch process) (Phase 3 Studies HGB-207, HGB-212 and long-term follow-up Study LTF-303). In current review, efficacy of beti-cel is based on Phase 3 study results. Results from Phase 1/2 study HGB-204 was included in exploratory analysis. Due to lack of drug product comparability information, Study #HGB-205 is not included in current review.

6.1 Study Design

The clinical pharmacology section of this BLA includes four clinical studies in which subjects with TDT were administered beti-cel with following sequence: Studies HGB-205, HGB-204, HGB-207 and HGB-212. Subjects who completed these studies were asked to enroll in a long-term follow-up Study LTF-303 (Table 1).

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.

¹ In current review, ZYNTGLO is referred to as beti-cel.

Table 1. Overview of Clinical Studies Evaluating Beti-cel in Subjects with TDT

Study Identifier (Status); Location of CSR or Protocol (as applicable)	Study Title	Number of Subjects with TDT and Genotype	Drug Product Characteristics and Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) from Study Protocol or Study Objective	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 (TDT or SCD) 4 TDT treated 4 TDT completed (all non- β^0/β^0)	Manufacturing Process 0 Drug product VCN criterion: 0.5 to 3.0 c/dg ^b $\geq 3.0 \times 10^6$ CD34+ cells/kg	4000 to 5200 $\mu\text{M} \cdot \text{min}^a$	RBC transfusion requirements (measured in milliliters [mL] per kilogram [kg]) per month and per year post-transplant. Number of total in-patient hospitalization days (post-transplant discharge) at 6, 12, and 24 months. Note: CSR included the same TI endpoint as detailed for the Phase 3 Studies HGB-207 and HGB-212	NA; study complete
HGB-204 (completed: 21 February 2018) 5.3.5.2 CSR HGB-204	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with 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)	18 planned 18 treated 18 completed (10 non- β^0/β^0 ; 8 β^0/β^0) 1 discontinued before conditioning	Manufacturing Process 1 Drug product VCN criterion: 0.3 to 5.0 c/dg ^b $\geq 3.0 \times 10^6$ CD34+ cells/kg	3600 to 5000 $\mu\text{M} \cdot \text{min}^a$	The sustained production of ≥ 2.0 g/dL of hemoglobin A (HbA) containing β^{A-T87Q} -globin for the 6 months between Month 18 and Month 24 post-transplant. Note: CSR included the same TI endpoint as detailed for the Phase 3 Studies HGB-207 and HGB-212	NA; study complete
HGB-207 (ongoing) 5.3.5.2 Interim CSR HGB-207	A Phase 3, Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β -Thalassemia, who do not have the β^0/β^0 Genotype, by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral β^{A-T87Q} -Globin Vector in Subjects ≤ 50 Years of Age	23 non- β^0/β^0 planned (15 for ≥ 12 and ≤ 50 years of age; 8 for < 12 years of age) 23 treated (all non- β^0/β^0) 19 completed 1 discontinued before conditioning	Manufacturing Process 2 Drug product VCN criterion: 0.8 to 6.6 c/dg ^b $\geq 5.0 \times 10^6$ CD34+ cells/kg	3800 to 4500 $\mu\text{M} \cdot \text{min}^c$	The proportion of subjects who meet the definition of “transfusion independence” (TI). TI is defined as a weighted average Hb ≥ 9 g/dL without any RBC transfusions for a continuous period of ≥ 12 months at any time during the study after drug product infusion.	09 March 2021

Study Identifier (Status); Location of CSR or Protocol (as applicable)	Study Title	Number of Subjects with TDT and Genotype	Drug Product Characteristics and Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) from Study Protocol or Study Objective	Data Cut-off Date for Ongoing Studies
HGB-212 (ongoing) 5.3.5.2 Interim CSR HGB-212	A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β -Thalassemia by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral β^{A-T87Q} -Globin Vector in Subjects ≤ 50 Years of Age	18 planned; must be β^0/β^0 , $\beta^0/IVS-I-110$, or $IVS-I-110/IVS-I-110$ genotype (at least 10 subjects < 18 years of age; at least 12 subjects without an $IVS-I-110$ mutation) 18 treated 10 completed 1 discontinued before conditioning	Manufacturing Process 2 Drug product VCN criterion: 0.8 to 6.6 c/dg ^b $\geq 5.0 \times 10^6$ CD34+ cells/kg	3800 to 4500 $\mu M \cdot min^c$	The proportion of subjects who meet the definition of “transfusion independence” (TI). TI is defined as a weighted average Hb ≥ 9 g/dL without any RBC transfusions for a continuous period of ≥ 12 months at any time during the study after drug product infusion.	09 March 2021
LTF-303 (ongoing) 5.3.5.2 Interim CSR LTF-303 (subjects with TDT only ^d)	A Longterm Follow-up of Subjects with Hemoglobinopathies Treated with Ex Vivo Gene Therapy Using Autologous Hematopoietic Stem Cells Transduced with Lentiviral Vector	Long-term follow-up for all subjects with a hemoglobinopathy who received drug product during bluebird bio-sponsored studies ^d 51 treated subjects with TDT enrolled (18 from Study HGB-204; 4 from Study HGB-205; 19 from Study HGB-207; 10 from Study HGB-212) 0 completed	Not applicable	Not applicable	Objective: Monitor for long-term safety and efficacy of the drug product Note: CSR included the same TI endpoint as detailed for the Phase 3 studies HGB-207 and HGB-212.	09 March 2021

Subjects with SCD (N = 3 treated in Study HGB-205) were not included in the pharmacodynamic analysis for this module.

^a Changed from 3200 to 4400 $\mu M \cdot min$ in Protocol HGB-205 Version 7.0 (19 May 2016) and in Protocol HGB-204 Version 3.1 (17 March 2014).

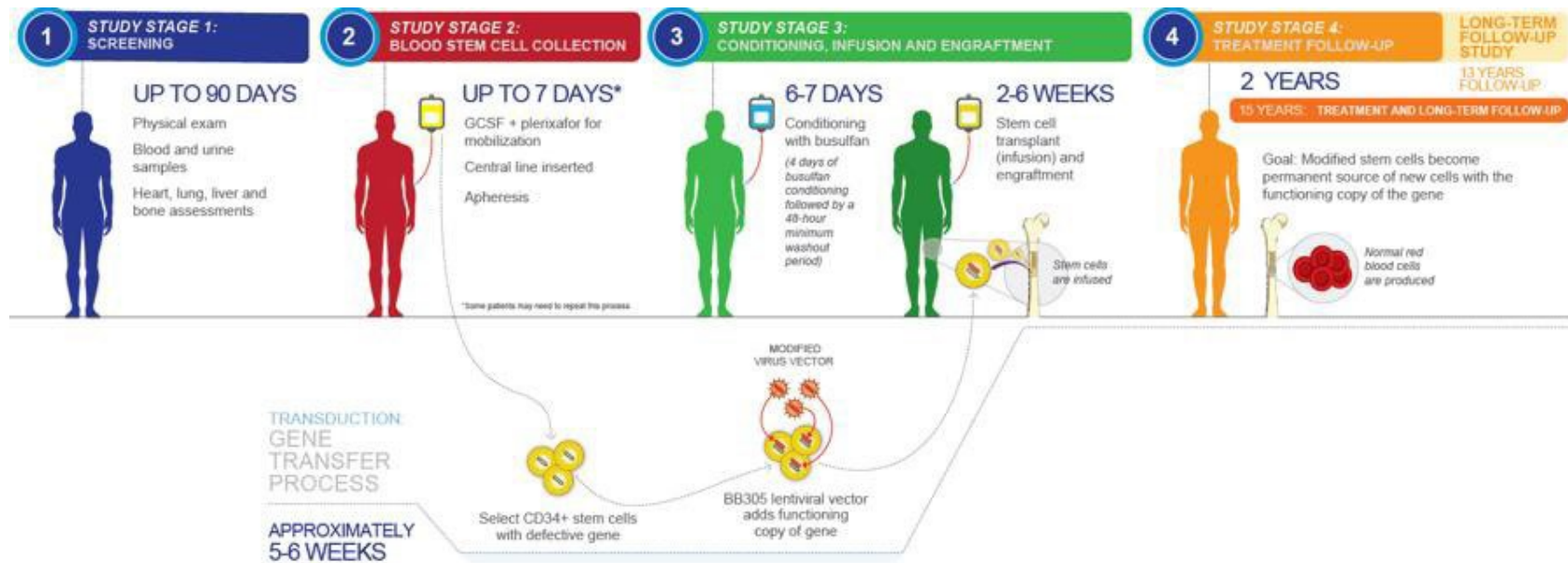
^b DP VCN was not specified per protocol; criterion given reflects the most recent manufacturing specifications used in each study for subjects with TDT.

^c Changed from 4000 to 5000 $\mu M \cdot min$ in Protocol HGB-207 Version 3.0 (19 June 2018) and in Protocol HGB-212 Version 2.0 (19 June 2018).

^d Subjects with SCD were not included in the pharmacodynamic analysis for this module or the interim CSR LTF-303.

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

Figure 1. Schematic of Study Design



Notes:

Periods estimated for each Study Stage are approximate.

Mobilization/apheresis procedure may need to be repeated to obtain sufficient cells for drug product manufacture.

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

The demography of subjects in all clinical studies is shown in Table 2.

Table 2. Demography of Clinical Study Subjects

	HGB-204 (N = 18)	HGB-207 (N = 23)	HGB-212 (N = 18)	All Phase 3 (N = 41)
Age at Informed Consent or Assent (years)				
N	18	23	18	41
Median	20.00	15.00	12.50	13.00
Min, Max	12.0, 35.0	4.0, 34.0	4.0, 33.0	4.0, 34.0
Age at Informed Consent or Assent (Category), n (%)^a				
≥ 18 Years	15 (83.3)	9 (39.1)	5 (27.8)	14 (34.1)
< 18 years	3 (16.7)	14 (60.9)	13 (72.2)	27 (65.9)
< 18 and ≥ 12 years	3 (16.7)	6 (26.1)	5 (27.8)	11 (26.8)
< 12 years	0	8 (34.8)	8 (44.4)	16 (39.0)
Sex, n (%)				
Male	5 (27.8)	11 (47.8)	10 (55.6)	21 (51.2)
Female	13 (72.2)	12 (52.2)	8 (44.4)	20 (48.8)
Race, n(%)				
Asian	14 (77.8)	13 (56.5)	7 (38.9)	20 (48.8)
White	4 (22.2)	8 (34.8)	10 (55.6)	18 (43.9)
Other ^b	0	2 (8.7)	0	2 (4.9)
Not Reported	0	0	1 (5.6)	1 (2.4)
Genotype, n (%)				
β^0/β^0	8 (44.4)	0	12 (66.7)	12 (29.3)
β^E/β^0	6 (33.3)	6 (26.1)	0	6 (14.6)
β^0/β^+	1 (5.6)	12 (52.2)	3 (16.7)	15 (36.6)
β^+/β^+	2 (11.1)	5 (21.7)	3 (16.7)	8 (19.5)
Other	1 (5.6)	0	0	0

^a Date of assent applicable for subjects <18 years old;

^b Reported as Asian Pakistani and Caucasian Father/Mother Thai

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

6.2 Dosing Characteristics

6.2.1 Busulfan

Prior to infusion of ZYNTGLO, busulfan was administered 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, with dose adjustment as needed based on PK monitoring. In Phase 3 studies, busulfan was administered at 0.8 mg/kg every 6 hours in children and adolescent to avoid higher peak concentrations. A daily busulfan target AUC of 4200 (3800 to 4500) $\mu\text{M}\cdot\text{min}$ was recommended in Phase 3 studies (Table 1). The median (min, max) value of estimated busulfan daily AUC was 4310.2 (3605, 9086) for Phase 3 (N=41) studies (Table 4).

Table 3. Relationships Between Busulfan Exposure and Engraftment (Neutrophil and Platelet)

X-axis (Independent)	Y-axis (Dependent)	Population^a	r value	p-value
Busulfan Average Daily AUC	Time to Neutrophil Engraftment	<i>All TDT</i>	r = 0.1476	p = 0.2485
		<i>All Phase 3</i>	r = 0.0427	p = 0.7909
		<i>All TDT</i>	r = 0.0971	p = 0.4564
		<i>All Phase 3</i>	r = 0.0427	p = 0.7909
	Time to Platelet Engraftment	<i>All TDT</i>	r = -0.0807	p = 0.5293
		<i>All Phase 3</i>	r = -0.0285	p = 0.8594
		<i>All TDT</i>	r = -0.1043	p = 0.4236
		<i>All Phase 3</i>	r = -0.0285	p = 0.8594

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

The relationship between conditioning busulfan AUC and engraftment was evaluated; busulfan AUC did not affect the kinetics of either neutrophil or platelet engraftment within the AUC range in the clinical studies in which subjects with TDT were treated with beti-cel (Table 3). This may be due to the fact that the AUC range of busulfan used in the clinical studies was narrow.

6.2.2 ZYNTEGLO (beti-cel)

ZYNTEGLO is a cell suspension for a single dose (one time treatment) intravenous (IV) infusion. The dose of CD34+ cells is based on accepted safe practice to achieve hematopoietic reconstitution with long-term engraftment after autologous transplantation. In the clinical practice of HSCs transplantation, a minimum dose of $\geq 1.5 \times 10^6$ cells/kg mobilized peripheral stem cells is associated with favorable engraftment kinetics. Lower cell doses may result in engraftment with delays in neutrophil and platelet recovery relative to higher doses. Preliminary results from Phase 1/2 studies suggest a minimum dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg to be used for pivotal Phase 3 studies (HGB-207 and HGB-212). The median (min, max) total cell dose of beti-cel of 9.40 (5.00, 42.10) $\times 10^6$ CD34+ cells/kg (N=41) was administered to subjects with TDT in Phase 3 studies (Table 3).

During the drug product development, drug product from three manufacturing processes were used in clinical studies: Process 0 (Study HGB-205), Process 1 (Study HGB-204), and Process 2 (Studies HGB-207, and HGB-212). Compared to Process 1, Process 2 includes an optimized (b) (4) step to (b) (4)

The (b) (4) DP VCN obtained using Process 2 was shown to be

associated with (b) (4), as well as (b) (4) VCN, in long-term-engrafting bone marrow cells.

The relationships between beti-cel dosing characteristics and PD responses are analyzed and discussed in Section 6.5.

Table 4. Dosing Characteristics of Beti-cel and Busulfan

Parameter	HGB-204 (N = 18)	HGB-207 (N = 23)	HGB-212 (N = 18)	All Phase 3 (N = 41)
Total DP Dose (CD34+ cells × 10⁶/kg)				
N	18	23	18	41
Median	8.10	8.10	10.75	9.40
Min, Max	5.20, 18.10	5.00, 19.90	5.90, 42.10	5.00, 42.10
DP VCN^a (weighted by cell dose; c/dg)				
N	18	23	18	41
Median	0.700	3.26	3.00	3.00
Min, Max	0.30, 1.50	1.90, 5.60	1.20, 7.00	1.20, 7.00
DP %LVV+ Cells^a (weighted by cell dose; %)				
N	18	23	18	41
Median	31.5	79.3	78.0	78.0
Min, Max	17, 58	34, 90	34, 94	34, 94
DP VCN/ DP%LVV+ Cells^a (vector copies per transduced cell)				
N	18	23	18	41
Median	2.350	4.44	3.71	4.00
Min, Max	1.52, 3.75	2.84, 7.65	2.59, 7.45	2.59, 7.65
Estimated Average Busulfan AUC (uM*min)				
N	18	23	18	41
Median	4092.2	4337.4	4236.8	4310.2
Min, Max	3030, 4714	3708, 8947	3605, 9086	3605, 9086

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

6.3 General Pharmacology

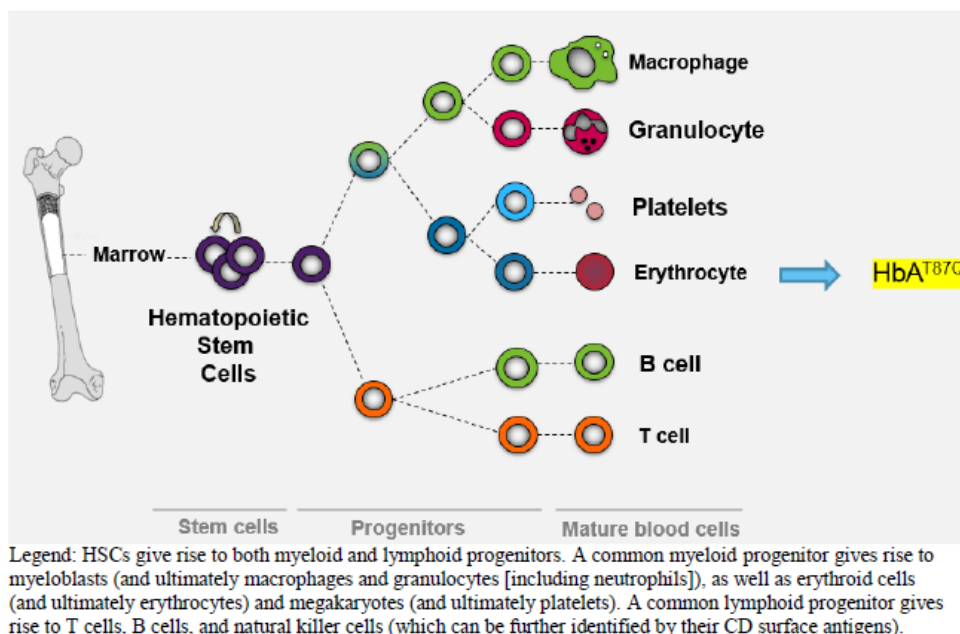
ZYNTEGLO (beti-cel) is an autologous gene therapy consisting of hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding β^{A-T87Q} -globin. Successfully transduced patient's HSCs incorporate the β^{A-T87Q} -globin gene into their genome, and therefore their progeny contain the transgene. Figure 2 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 the peripheral blood, only cells within the erythroid lineage will produce the transcription factors required to drive expression of the 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 will express the 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. The presence of β^{A-T87Q} -globin is detected using RP-HPLC.

After infusion of ZYNTEGLO (beti-cel), transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce red blood cells (RBCs) containing biologically active β^{A-T87Q} -globin that will combine with α -globin to produce functional Hb containing β^{A-T87Q} -globin (HbA^{T87Q}).

Based on the nature of ZYNTEGLO (beti-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 ZYNTEGLO (beti-cel), pharmacodynamic (PD) parameters were measured to detect the presence of integrated proviral sequences and the expression of transgene in differentiated cells. In addition, ZYNTEGLO (beti-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 2. Hematopoietic Stem Cell Differentiation



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

6.4 Pharmacodynamics of Beti-cel

Successful treatment with beti-cel 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 beti-cel 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 beti-cel, an autologous gene therapy consisting of HSCs that have been genetically modified ex vivo and is intended for a single dose IV infusion.

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

PB VCN levels were measured using qPCR method. After infusion of beti-cel, PB VCN levels increased rapidly over the first few months before reaching a plateau (Figure 3 & Table 5). At

Month 6, the median (min, max) PB VCN levels in the Phase 3 studies was 1.29 (0.16, 4.52) c/dg (N=37). PB VCN levels generally remained stable as of the data cut off data of all studies. All treated subjects had detectable beti-cel LVV sequences in the peripheral blood through last follow-up. High inter-subject variability of PB VCN kinetic profiles was observed.

Figure 3. Vector copy number over time in peripheral blood (Phase 3 Studies)

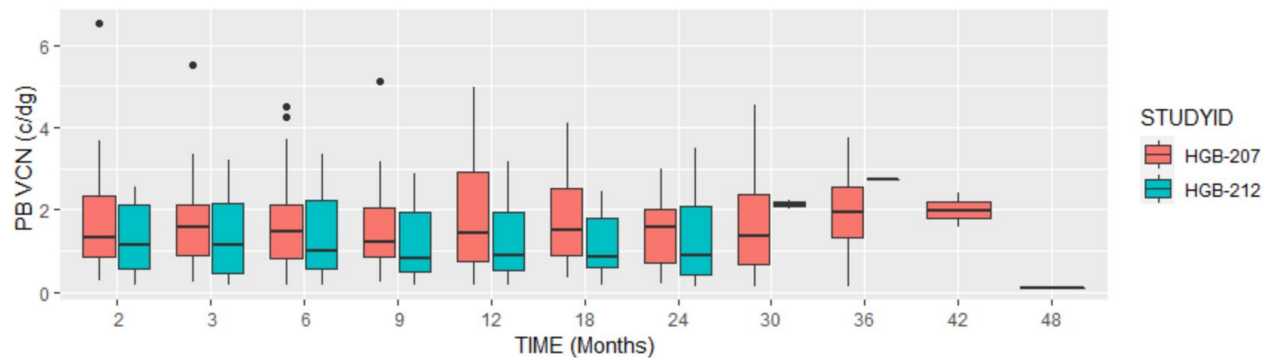


Table 5. Summary of Vector Copy Number in Peripheral Blood (PB VCN)

	Phase 3 Studies		
Parameter Visit	HGB-207 (N = 23)	HGB-212 (N = 18)	All Phase 3 (N = 41)
DP VCN (c/dg)^a			
N	23	18	41
Median	3.26	3.00	3.00
Min, Max	1.90, 5.60	1.20, 7.00	1.20, 7.00
PB VCN (c/dg)			
Month 6			
N	22	15	37
Median	1.40	0.88	1.29
Min, Max	0.18, 4.52	0.16, 3.33	0.16, 4.52
Month 24			
N	20	10	30
Median	1.62	0.90	1.43
Min, Max	0.20, 3.00	0.14, 3.49	0.14, 3.49
Month 36			
N	8	1	9
Median	1.93	2.73	2.19
Min, Max	0.13, 3.74	2.73, 2.73	0.13, 3.74
Month 48			
N	1	0	1
Median	0.11, 0.11	-	0.11, 0.11
Min, Max			

Month 60			
N	0	0	0
Median	-	-	-
Min, Max	-, -	-, -	-, -
Year 6			
N	0	0	0
Median	-	-	-
Min, Max	-, -	-, -	-, -

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

PB VCN Levels in Special Populations

The potential impact of intrinsic factors on PB VCN levels were evaluated using population PD modeling analysis with data from Studies HGB-204, HGB-207 and HGB-212. 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 time to steady-state for PB VCN.

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

After infusion of beti-cel, 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 4 shows the kinetic profiles of HbA^{T87Q} in peripheral blood for subjects with TDT. The kinetic profiles of HbA^{T87Q} in peripheral blood were similar among all subjects. In general, HbA^{T87Q} increased steadily after beti-cel infusion and stabilized by approximately Month 6 after infusion. High inter-subject variability was observed for HbA^{T87Q} levels at any specific time point.

HbA^{T87Q} levels in peripheral blood in Phase 1/2 studies were lower than that in Phase 3 studies. At Month 6, subjects had a median (min, max) HbA^{T87Q} of 4.56 (0.43, 8.89) g/dL in the Phase 1/2 study (HGB-204, N=18) and of 8.737 (0.00, 12.01) g/dL in Phase 3 studies, HGB-207 and HGB-212 (N = 35) (Table 6).

HbA^{T87Q} in the Phase 1/2 study, HGB-204 continued to remain durable at last follow-up through Year 6, and through Month 36 in the Phase 3 studies, indicating sustained expression of the β^{A-T87Q} protein derived from irreversible integration of the β^{A-T87Q} -globin gene into long-term HSCs (Section 7.1 Table 13).

Figure 4. HbAT87Q over time in peripheral blood (Phase 3 Studies)

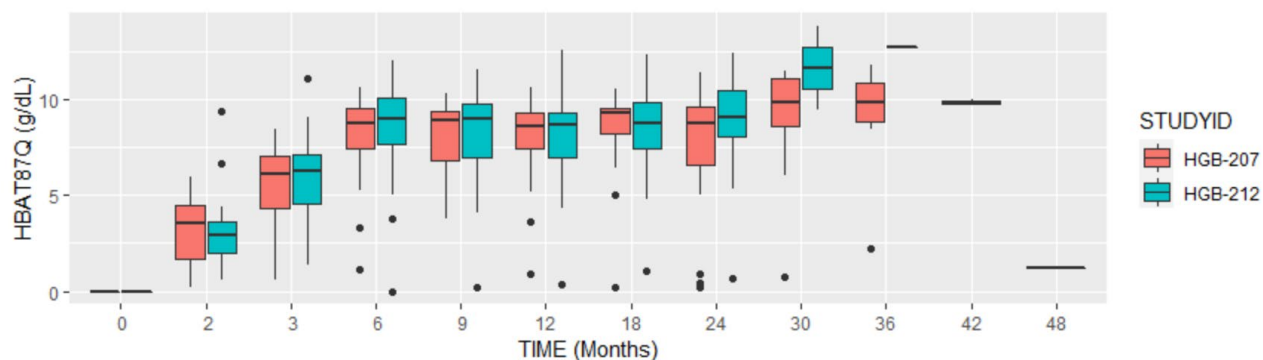


Table 6. Summary of HbA^{T87Q} in Peripheral Blood

HbA ^{T87Q} (g/dL)	HGB-207 (N = 23)	HGB-212 (N = 18)	All Phase 3 (N = 41)
Visit			
Month 6			
N	20	15	35
Median	8.733	8.934	8.737
Min, Max	1.12, 10.60	0.00, 12.01	0.00, 12.01
Month 24			
N	20	10	30
Median	8.802	9.087	8.802
Min, Max	0.34, 11.40	0.71, 12.43	0.34, 12.43
Month 36			
N	8	1	9
Median	9.857	12.724	10.622
Min, Max	2.23, 11.79	12.72, 12.72	2.23, 12.72
Month 48			
N	1	0	1
Median	1.232	-	1.232
Min, Max	1.23, 1.23	-, -	1.23, 1.23
Month 60			
N	0	0	0
Median	-	-	-
Min, Max	-, -	-, -	-, -
Year 6			
N	0	0	0
Median	-	-	-
Min, Max	-, -	-, -	-, -

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

Reviewer's Comments:

Only one subject had HbA^{T87Q} values at Month 48. This subject was one of the 4 subjects in the ongoing Ph3 studies who did not achieve TI.

HbA^{T87Q} Levels in Special Populations

The potential impact of intrinsic factors on HbA^{T87Q} levels were evaluated using population PD modeling analysis with data from Studies HGB-204, HGB-207 and HGB-212. 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 time to steady-state for HbA^{T87Q}.

6.4.3 Hemoglobin Fractions in Peripheral Blood

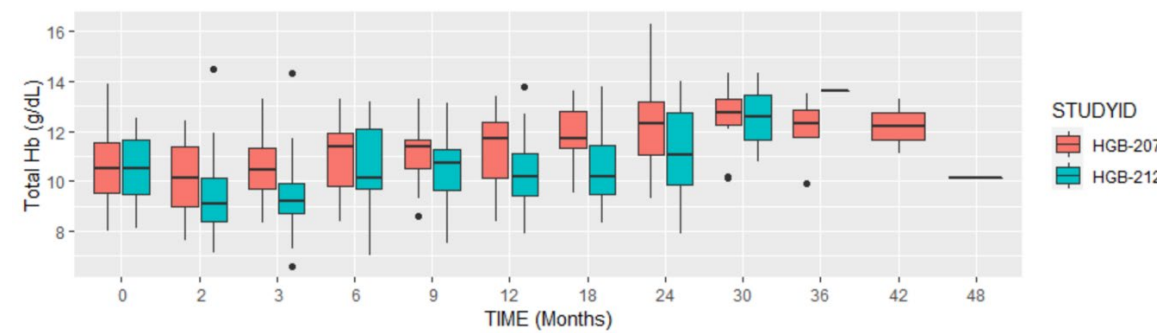
Endogenous erythroid cells in subjects produce multiple types of Hb. Normal adults generally have Hb primarily composed of α -globin complexed with β -globin (to form HbA, approximately 95% of total Hb), with lower amounts of α -globin complexed with either δ -globin (to form HbA₂, 1.5% to 3.5% of total Hb), or with γ G- or γ A-globin (to form HbF, < 2% of total Hb). Subjects with TDT still produce endogenous HbA₂ and HbF, and depending on type of β -globin mutation, they produce either no endogenous HbA (if β^0/β^0 genotype), low amounts of endogenous HbA (if β^+ mutation that is not β^E), or HbE (if β^E mutation). Erythroid cells derived from transduced HSCs also produce HbA^{T87Q}.

The total unsupported Hb and Hb fractions over time were evaluated. In general, HbAT87Q was the major contributor to unsupported total Hb. The relative contributions of endogenous Hb may differ for each individual subjects.

Unsupported Total Hb:

After administration of beti-cel, unsupported total Hb levels had an initial decrease followed elevation to reach a plateau around Month 6. At Month 6, the median (min, max) unsupported total Hb levels were 11.55 (8.4, 13.3) g/dL (N=22) and 10.20 (8.5, 13.2) g/dL (N=15) in Study 207 and 212 respectively. The median unsupported total Hb levels were > 10 g/dL during the observation period of Phase 3 studies.

Figure 5. Total Unsupported Hb Over Time (Phase 3 Studies)



6.4.3.1 Unsupported Endogenous Hb Fractions

Figure 5 shows the kinetic profiles of unsupported endogenous Hb fractions (at least 60 after any prior pRBC transfusion) in Phase 3 studies subjects who had stopped receiving pRBC transfusions after receiving beti-cel.

Total unsupported endogenous Hb: after infusion of beti-cel, the total unsupported endogenous Hb levels had an initial decrease followed by a plateau by Month 6 to 9 (Figure 6 A). The initial decrease of total unsupported endogenous Hb levels is due to metabolism of any exogenous RBCs remaining from pRBC transfusions. At the plateau levels reflect the endogenous Hb production in those subjects.

Unsupported HbA: the kinetic profiles of unsupported endogenous HbA were evaluated in subjects with at least one β^+ allele that is not β^E mutation. HbA levels initially decrease at early times after drug product infusion in subjects with unsupported total Hb values, as exogenous RBCs are metabolized, and then HbA levels plateau by Months 6 to 9, reflecting endogenous HbA production in those subjects (Figure 6 B). Median (min, max) unsupported HbA was 0.78 (0.063, 2.90; N = 21) g/dL at Month 6 in subjects with a non- β^0/β^0 genotype.

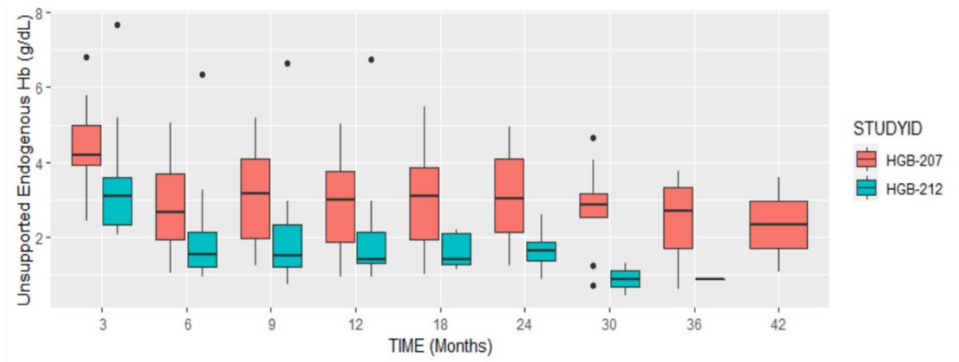
Unsupported HbA2: unsupported HbA2 levels initially increased and reached the plateau around Month 6 to 9 post infusion of beti-cel (Figure 6 C). HbA2 contributions to the total Hb levels were relatively low. Median (min, max) unsupported HbA₂ was 0.42 (0.24, 0.94; N = 35) g/dL at Month 6 in Phase 3 study subjects.

Unsupported HbE: after beti-cel infusion, the kinetic profiles of HbE were similar to those of HbA^{T87Q}, with endogenous levels of HbE increasing as the hematopoietic system is repopulated after drug product infusion, reaching plateau levels by Months 6 to 9 (Figure 6 D). The median (min, max) unsupported HbE that was produced in subjects with at least one β^E allele in Study HGB-207 was 2.44 (2.21, 2.79; N = 5) g/dL at Month 6.

Unsupported HbF: HbF levels showed a peak around 2 to 3 months after drug product infusion, that may reflect a skewing of endogenous hematopoiesis towards fetal Hb during reconstitution, before plateauing at approximately Month 12 (Figure 6 E). Median (min, max) unsupported HbF was 0.63 (0.18, 5.95; N = 35) g/dL at Month 6 in Phase 3 study subjects.

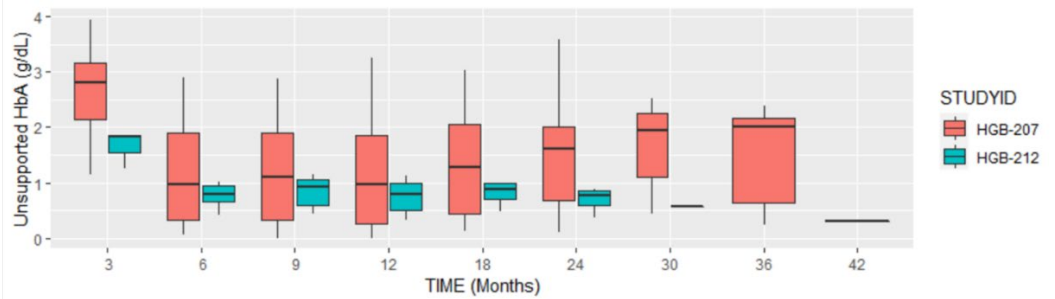
Figure 6. Total Unsupported Endogenous Hb Fractions Over Time (Phase 3 Studies)

A. Total unsupported endogenous Hb over time



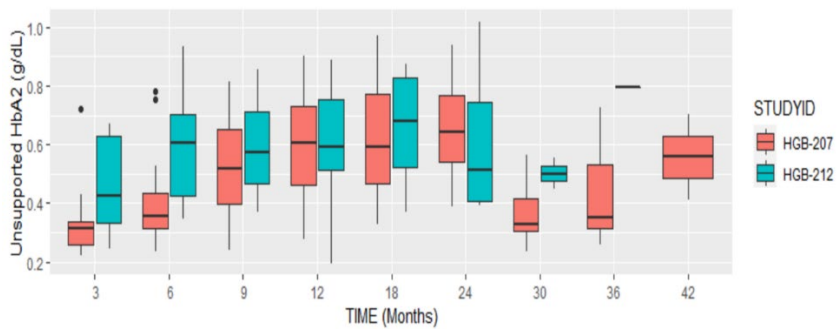
Number of Subjects										
207	13	19	21	22	19	17	11	7	2	
212	12	14	13	14	10	9	2	1		

B. Unsupported HbA (subjects with at least one β^+ allele)



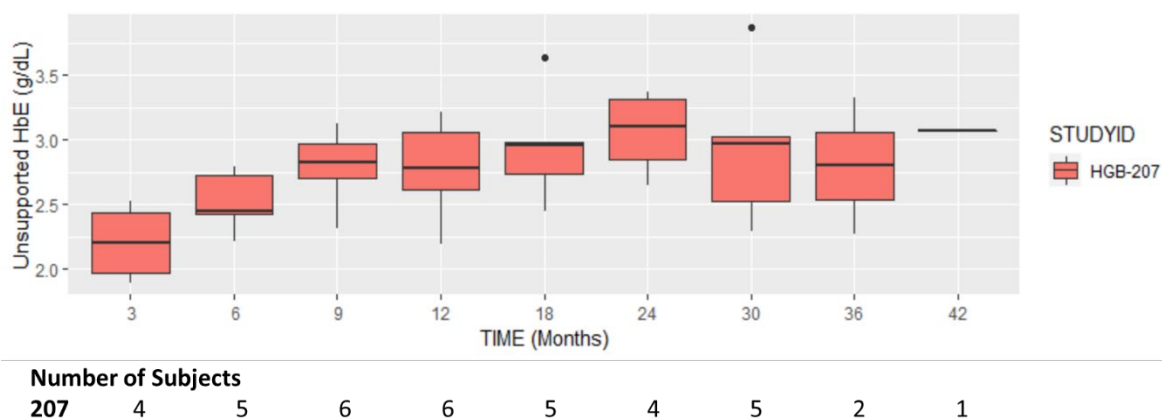
Number of Subjects										
207	9	14	15	16	14	13	6	5	1	
212	3	5	5	6	4	4	1			

C. Unsupported HbA2

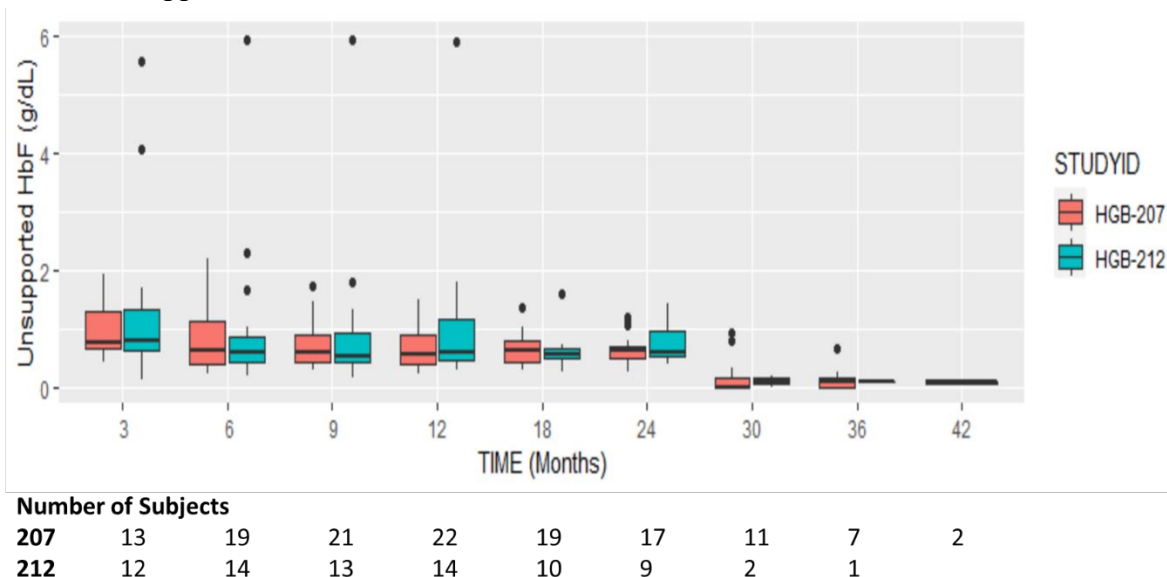


Number of Subjects										
207	13	19	21	22	19	17	11	7	2	
212	12	14	13	14	10	9	2	1		

D. Unsupported HbE (subjects with at least one β^{Emi})



E. Unsupported HbF



6.5 Beti-cel Drug Product Dosing Characteristics and Pharmacodynamic Responses, Clinical Outcomes

ZYNTÉGLO (beti-cel) 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 heterogeneity of beti-cel 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 PD Responses

During the drug development of beti-cel, drug product from three manufacturing processes were used in clinical studies: Process 0 (Study HGB-205), Process 1 (Study HGB-204), and Process 2

(Studies HGB-207, and HGB-212). Compared to Process 1, Process 2 includes optimized

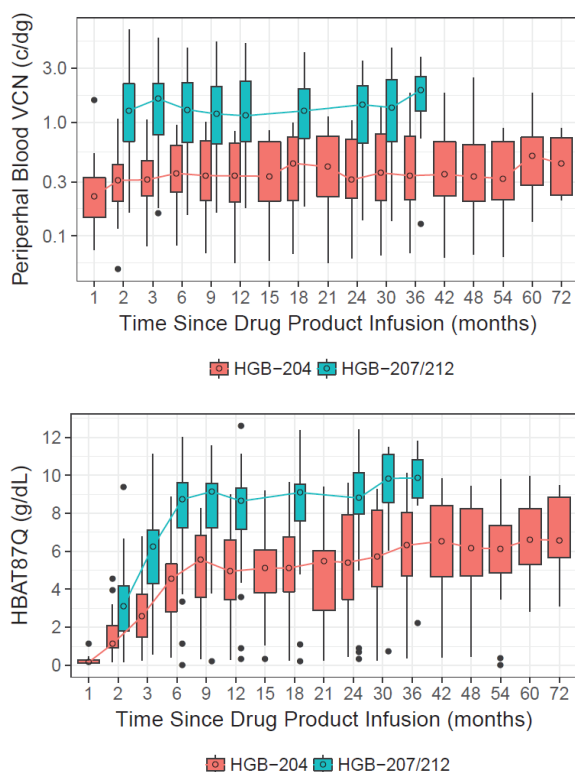
(b) (4) step to (b) (4)

The (b) (4) DP VCN obtained using Process 2 was shown to be associated with (b) (4)

The PD responses of beti-cel from different manufacturing processes were assessed. As shown in Figure 7, after administration of beti-cel, the median PB VCN and HbA^{T87Q} values in Phase 3 studies (Studies # HGB-207 and HGB-212) were higher than that in Phase 1/2 study (Study HGB-204). The median DP VCN (min, max) were 0.70 (0.30, 1.50) and 3.00 (1.20, 7.00) for the Phase 1/2 study (Study HGB-204) and Phase 3 studies (Studies # HGB-207 and HGB-212). The median DP %LVV+ Cells (min, max) were 31.5 (17.0, 58.0) and 78.0 (34.0, 94.0) for the Phase 1/2 study (Study HGB-204) and Phase 3 studies (Studies # HGB-207 and HGB-212).

HbA^{T87Q} levels in peripheral blood in Phase 1/2 studies were lower than that in Phase 3 studies (Figure 7). At Month 6, subjects had a median (min, max) HbA^{T87Q} of 4.56 (0.43, 8.89) g/dL in the Phase 1/2 study (HGB-204, N=18) and of 8.74 (0.00, 12.01) g/dL in Phase 3 studies, HGB-207 and HGB-212 (N = 35).

Figure 7. PB VCN and HbAT87Q by Study and Manufacturing Process



Source: Applicant. Module 5, section 5.3.4.2. 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 Beti-Cel Dosing Characteristics and Pharmacodynamic Responses

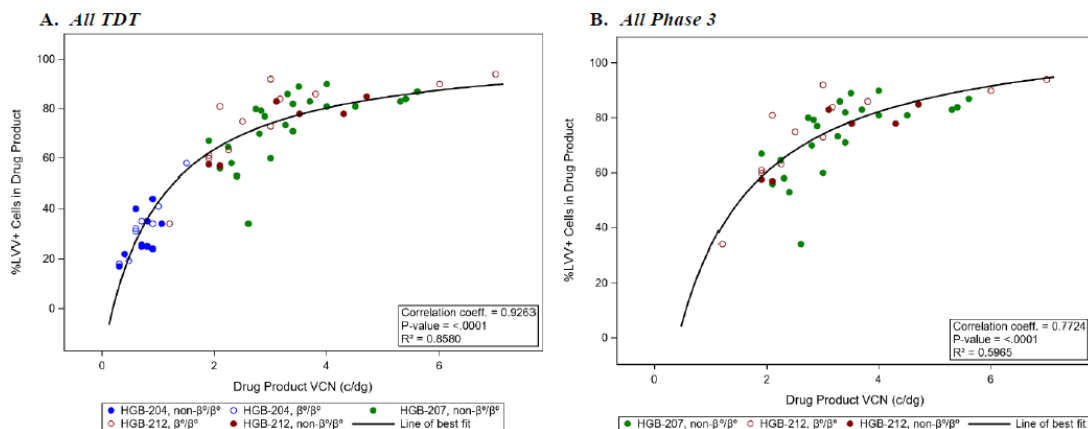
X-axis (Independent)	Y-axis (Dependent)	Population^a	r value	p-value
DP VCN	DP %LVV+ Cells	<i>All TDT</i> <i>All Phase 3</i>	r = 0.9263 r = 0.7724	p = <0.0001 p = <0.0001
	PB VCN at M6	<i>All TDT</i> <i>All Phase 3</i>	r = 0.6438 r = 0.6225	p = <0.0001 p = <0.0001
	HbA ^{T87Q} at M6	<i>All TDT</i> <i>All Phase 3</i>	r = 0.6804 r = 0.5068	p = <0.0001 p = 0.0019
	HbA ^{T87Q} at M24	<i>All TDT</i> <i>All Phase 3</i>	r = 0.5605 r = 0.4501	p = <0.0001 p = 0.0126
DP %LVV+ Cells	PB VCN at M6	<i>All TDT</i> <i>All Phase 3</i>	r = 0.8199 r = 0.7952	p = <0.0001 p = <0.0001
	HbA ^{T87Q} at M6	<i>All TDT</i> <i>All Phase 3</i>	r = 0.7557 r = 0.6204	p = <0.0001 p = <0.0001
	HbA ^{T87Q} at M24	<i>All TDT</i> <i>All Phase 3</i>	r = 0.6437 r = 0.5907	p = <0.0001 p = 0.0006
Total Cell Dose	HbA ^{T87Q} at M6	<i>All TDT</i> <i>All Phase 3</i>	r = -0.0531 r = -0.2006	p = 0.6948 p = 0.2480
	HbA ^{T87Q} at M24	<i>All TDT</i> <i>All Phase 3</i>	r = -0.1809 r = -0.4541	p = 0.1994 p = 0.0117

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

DP VCN versus DP %LVV+ Cells

Both DP VCN and DP %LVV+ cells measure drug product characteristics related to transduction efficiency. DP VCN relates to both the number of copies per cell and the percentage of transduced cells in the drug product. DP %LVV+ cells reflect the percentage of transduced cells in drug product. 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 plateaued at higher levels of DP VCN after reaching approximately 80% to 90% DP %LVV+ cells (Figure 8).

Figure 8. DP VCN Versus DP %LVV+ Cells



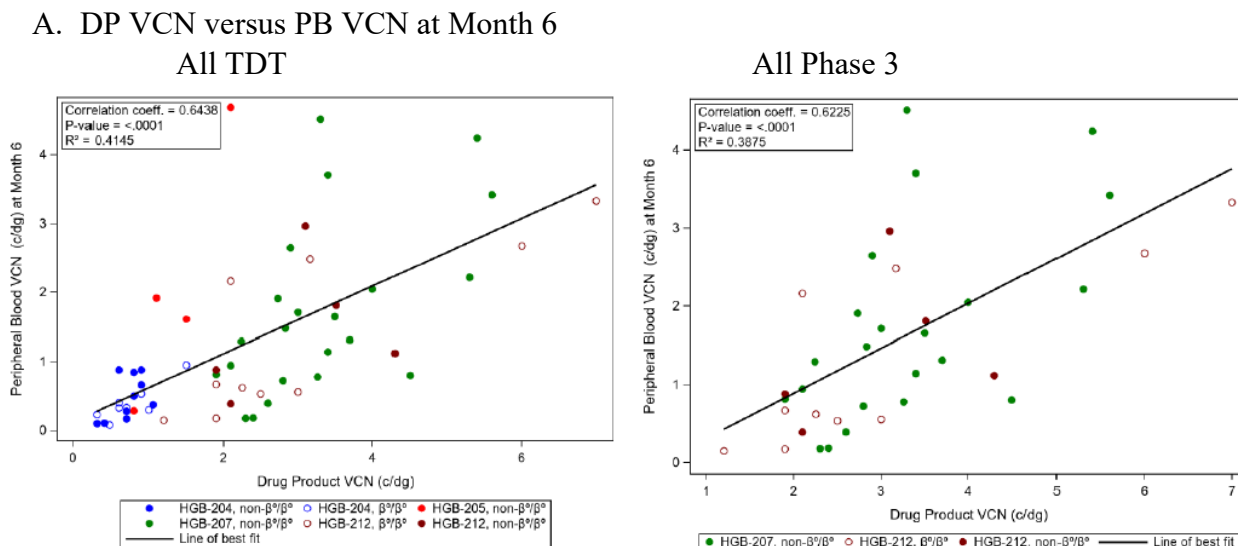
Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

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

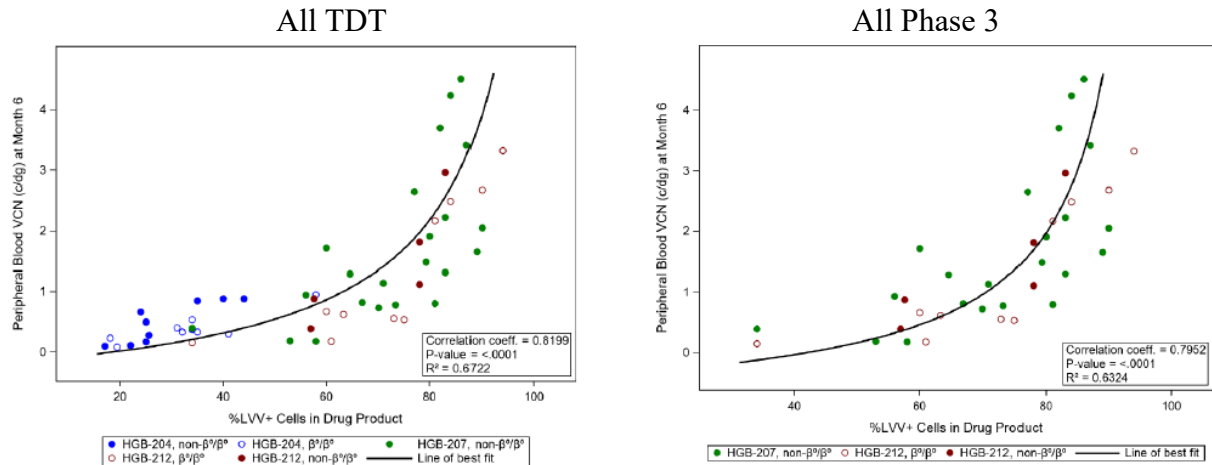
As shown in Figure 9 A, positive linear relationship was identified between drug product characteristics, DP VCN and PB VCN: treatment with beti-cel with higher DP VCN resulted in higher PB VCN levels ($p < 0.0001$).

Positive correlation was also observed between DP %LVV+ cells and PB VCN levels at Month 6 post beti-cel infusion. As shown in Figure 9 B, PB VCN increased approximately linearly as DP %LVV+ Cells increased up to approximately 80%, after which there are several subjects who have much higher PB VCNs than expected for a linear relationship. Population PD analysis also indicated that DP %LVV+ cells was the most important covariate.

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



B. DP %LVV+ cells versus PB VCN at Month 6



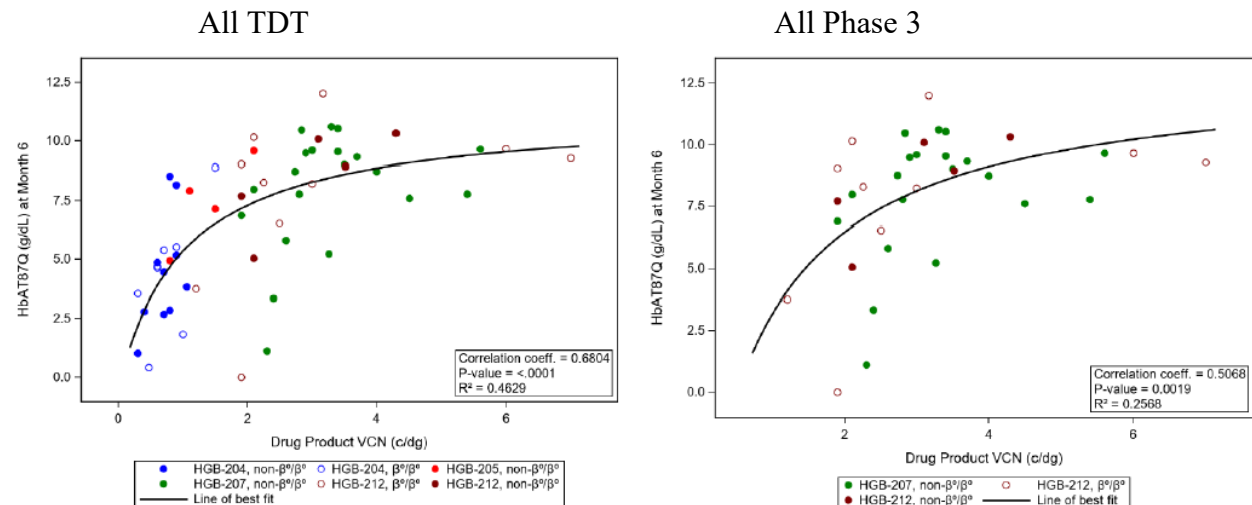
Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

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

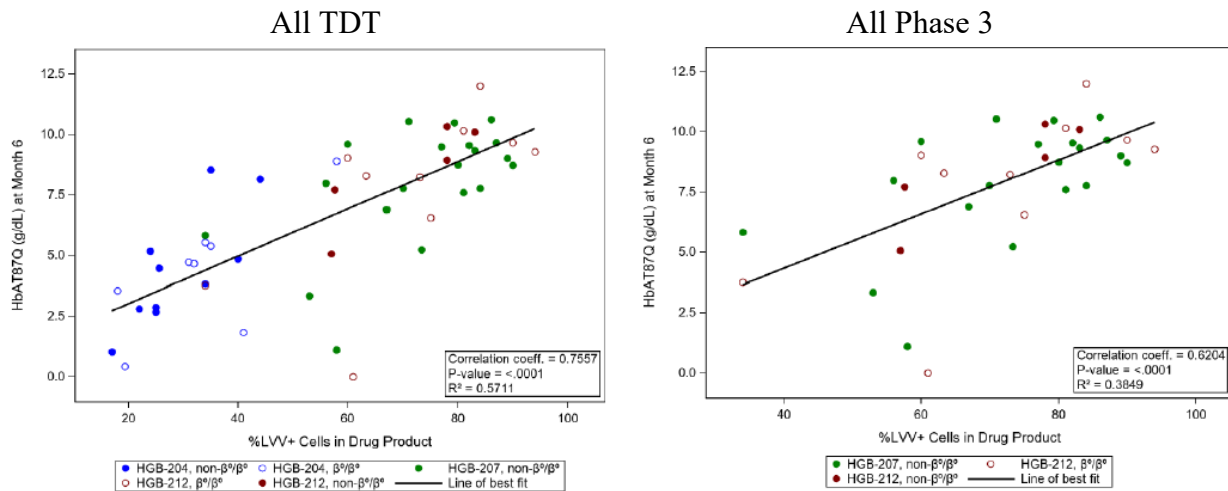
As depicted in Figure 10A and Table 7, there was a significant correlative relationship between DP VCN and HbA^{T87Q} levels at Month 6 after infusion of beti-cel: HbA^{T87Q} increased gradually with DP VCN at lower DP VCN values, but at higher DP VCN values, HbA^{T87Q} levels plateaued at approximately 8 to 10 g/dL. This observation indicates that β-globin (or total Hb) levels within erythroid cells may be regulated below some upper levels: the excess β-globin is either metabolized or further production is down-regulated.

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

A. DP VCN versus HbA^{T87Q} at Month 6



B. DP %LVV+ cells versus HbA^{T87Q} at Month 6



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

There was a significant correlative linear relationship between DP %LVV+ cells and HbA^{T87Q} at Month 6 (correlative coefficient = 0.7557, $p < 0.001$) (Figure 10 B and Table 7). Subjects received beti-cel with higher DP %LVV+ cells had higher HbA^{T87Q} at Month 6, compared to subjects received beti-cel with lower DP %LVV+ cells.

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

The relationship between total cell dose of beti-cel and HbA^{T87Q} in peripheral blood at early and late time points (Month 6 and Month 24) was assessed. No correlation was observed between total CD34+ cell dose and HbA^{T87Q} in peripheral blood at either Month 6 or Month 24 for the *All TDT* pool, indicating that the lowest cell dose evaluated to date was adequate for effective reconstitution of HSCs in treated subjects (Table 7). A potential negative correlation between total CD34+ cell dose and HbA^{T87Q} in peripheral blood at Month 24 was observed, but not at Month 6. To assess potential impact on clinical outcome – transfusion independence, this reviewer analyzed the relationship between total CD34+ cell dose and total unsupported Hb in peripheral blood at Month 24, and no correlation was identified.

6.5.1.4 Beti-cel Different Cell Subpopulations versus PB VCN and HbA^{T87Q}

The impact of different cell subpopulations, such as long-term repopulating cells and short-term progenitor cells, on PD parameters was also evaluated. No correlation between beti-cel subpopulations and PB VCN and HbAT87Q at post-infusion Month 6 and 24 were observed.

6.5.2 DP Dosing Characteristics and Clinical Outcomes

6.5.2.1 Transfusion Independence (TI)

The relationships between beti-cel dosing characteristics (DP VCN, DP %LVV+ Cells, total cell dose, and HbA^{T87Q} expression) and clinical efficacy outcome, transfusion independence was evaluated using logistic regression analysis. Univariate logistic analysis results suggest total cell dose and β^{A-T87Q} -globin quantitative protein expression may correlate with TI. Multi-variables logistic regression analysis showed potential correlation between β^{A-T87Q} -globin quantitative protein expression and TI (Table 8).

Table 8. Multivariate Logistic Regression Analysis for Dosing Characteristics of Beti-cel and Transfusion Independence (Phase 3 Studies)

Covariates/Attributes	Estimate	Standard Error	P value
Intercept	-10.22	8.05	0.2042
Total Cell Dose	-0.16	0.29	0.5872
β^{A-T87Q} -globin quantitative protein expression	0.06	0.03	0.0466 *

6.5.2.2 Neutrophil and platelet engraftment

The relationship between total cell dose of beti-cel and engraftment (the day of engraftment for either neutrophil or platelet) was evaluated (Table 9). No correlation was identified between beti-cel total cell dose and either neutrophil or platelet engraftment. The results suggest the lowest total cell dose of beti-cel investigated in the clinical studies was adequate for effective reconstitution of HSCs in subjects treated with beti-cel.

Table 9. Relationships Between Beti-Cel Dosing Characteristics and Engraftment (Neutrophil and Platelet)

X-axis (Independent)	Y-axis (Dependent)	Population ^a	r value	p-value
Total Cell Dose	Time to Neutrophil Engraftment	All TDT All Phase 3	r = 0.2214 r = 0.2307	p = 0.0812 p = 0.1466
	Time to Platelet Engraftment	All TDT All Phase 3	r = -0.0308 r = 0.0220	p = 0.8104 p = 0.8916

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

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 10 and discussed below.

Table 10. Relationships between Pharmacodynamic Parameters

X-axis (Independent)	Y-axis (Dependent)	Population ^a	r value	p-value
PB VCN at M6	PB VCN at M24	<i>All TDT</i> <i>All Phase 3</i>	r = 0.9379 r = 0.9226	p = <0.0001 p = <0.0001
	HbA ^{T87Q} at M6	<i>All TDT</i> <i>All Phase 3</i>	r = 0.8681 r = 0.8222	p = <0.0001 p = <0.0001
PB VCN at M24	HbA ^{T87Q} at M24	<i>All TDT</i> <i>All Phase 3</i>	r = 0.8364 r = 0.8022	p = <0.0001 p = <0.0001
HbA ^{T87Q} at M6	HbA ^{T87Q} at M12	<i>All TDT</i> <i>All Phase 3</i>	r = 0.9174 r = 0.9611	p = <0.0001 p = <0.0001
	HbA ^{T87Q} at M24	<i>All TDT</i> <i>All Phase 3</i>	r = 0.9316 r = 0.9606	p = <0.0001 p = <0.0001
Unsupported Total Hb at M6	Unsupported Total Hb at M12	<i>All TDT</i> <i>All Phase 3</i>	r = 0.8928 r = 0.9409	p = <0.0001 p = <0.0001
	Unsupported Total Hb at M24	<i>All TDT</i> <i>All Phase 3</i>	r = 0.8916 r = 0.8848	p = <0.0001 p = <0.0001
Unsupported Endogenous Hb at M6	HbA ^{T87Q} at M6	<i>All TDT</i> <i>All Phase 3</i>	r = -0.7523 r = -0.7698	p = <0.0001 p = <0.0001
	Unsupported Total Hb at M6	<i>All TDT</i> <i>All Phase 3</i>	r = -0.1893 r = -0.1849	p = 0.2078 p = 0.3030
Unsupported Endogenous Hb at M12	HbA ^{T87Q} at M12	<i>All TDT</i> <i>All Phase 3</i>	r = -0.7019 r = -0.6182	p = <0.0001 p = <0.0001
	Unsupported Total Hb at M12	<i>All TDT</i> <i>All Phase 3</i>	r = -0.0544 r = 0.0765	p = 0.7077 p = 0.6625

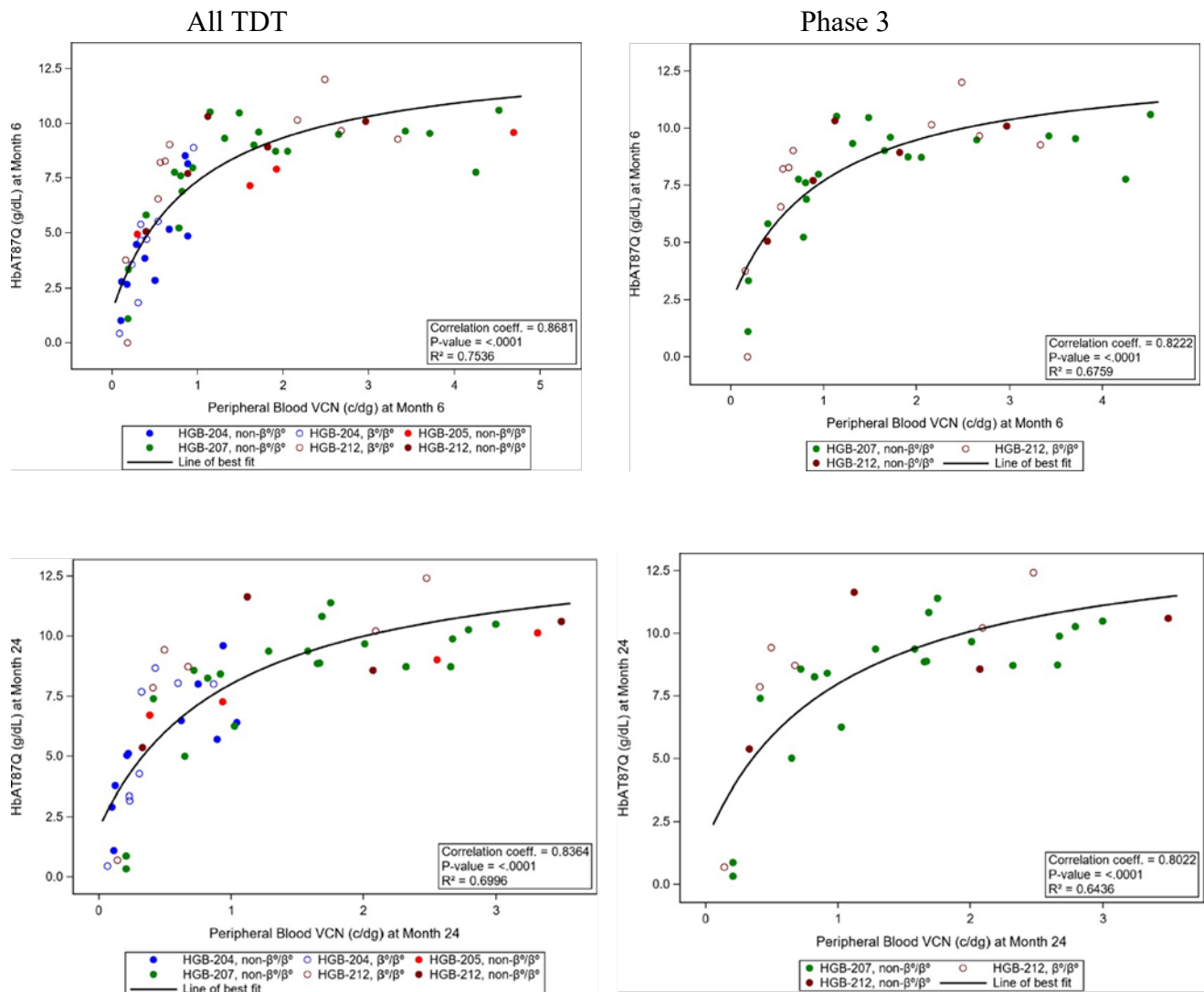
Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

After infusion of beti-cel, PB VCN and HbA^{T87Q} generally increased steadily to reach a plateau. For both PB VCN and HbA^{T87Q}, correlation was observed between Month 6 and Month 24 levels.

PB VCN versus HbA^{T87Q}

Figure 11 shows the relationships between PB VCN and HbA^{T87Q} at Month 6 and Month 24. HbA^{T87Q} increased quickly with the increase of PB VCN at lower PB VCN levels, followed by an HbA^{T87Q} plateau at higher PB VCN levels. This observation suggests the regulation of β -globin levels within erythroid cells and selection of erythroid cells producing β^{A-T87Q} -globin during engraftment for balanced α -globin/ β -globin ratios.

Figure 11. Relationships between PB VCN and HbA^{T87Q} at Month 6 and Month 24

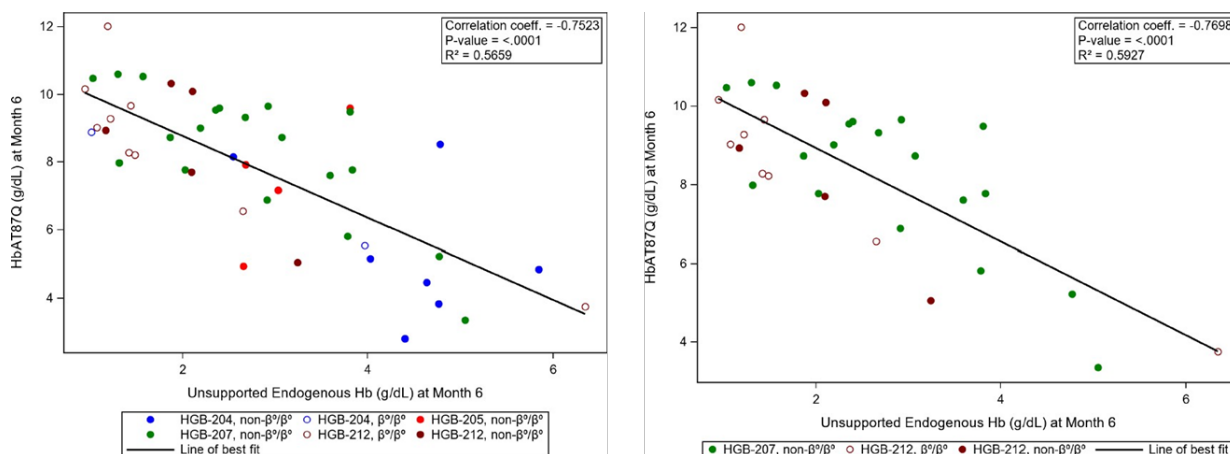


Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

Unsupported Endogenous Hb versus HbA^{T87Q} and Unsupported Total Hb

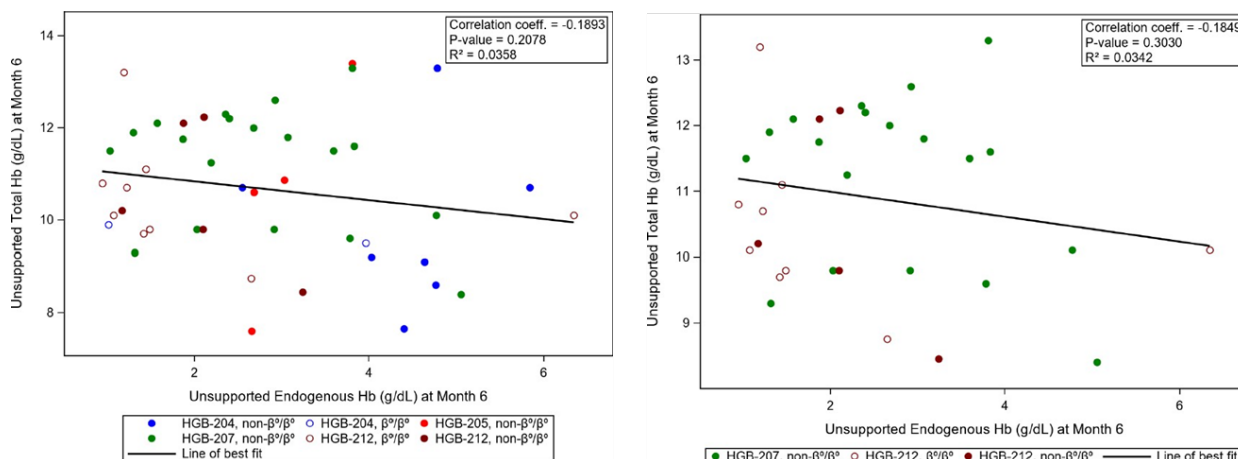
The potential correlations between unsupported endogenous Hb and HbA^{T87Q} as well as unsupported total Hb were assessed. A negative correlation between unsupported endogenous Hb and HbA^{T87Q} levels was observed (Table 10 & Figure 12). There's no correlation between unsupported endogenous Hb and unsupported total Hb identified (Table 10 & Figure 13). Above observations suggest a feedback mechanism regulating both endogenous β -globin and transgenic β^{A-T87Q} -globin.

Figure 12. Relationships between Unsupported Endogenous Hb at Month 6 and HbA^{T87Q} at Month 6



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

Figure 13. Relationships between Unsupported Endogenous Hb at Month 6 and Unsupported Total Hb at Month 6



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

6.6.2 PD responses and Transfusion Independence (TI)

The efficacy of beti-cel was established based on achievement of transfusion independence (TI), which is defined as a weighted average Hb ≥ 9 g/dL without any pRBC transfusions for a continuous period of ≥ 12 months at any time during the study, after infusion of beti-cel.

The Applicant performed analysis using contingency table (Table 11) to assess relationships between unsupported total Hb and TI status with Phase 3 studies data. Among 31 subjects who had ≥ 9 g/dL unsupported total Hb at Month 6, all of them achieved TI. The Fisher's exact test showed statistically significant association between unsupported total Hb levels and TI ($p < 0.0001$), and the Kappa statistics results also suggested an agreement ($k=0.8732$).

Table 11. Contingency Table of Unsupported Total Hb at Month 6 versus Transfusion Independence (TI) (Phase 3 Studies)

	N	Achieved TI	Did Not Achieve TI
Do Not Have Unsupported Total Hb ≥ 9 g/dL at Month 6	5	1	4
Unsupported Total Hb ≥ 9 g/dL at Month 6	31	31	0
Total TI-evaluable subjects	36	32	4

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

To further explore the relationships between PD parameters (HbA^{T87Q} and unsupported total Hb in peripheral blood) with transfusion status, the reviewer compared PD values at each time point with the transfusion status in the Phase 3 studies. Subjects with higher HbA^{T87Q} levels were less likely to need blood transfusion. The median (min, max) HbA^{T87Q} at the time when no blood transfusion was needed was 8.44 (0.75, 13.85) g/dL and the median (min, max) HbA^{T87Q} at the time that subjects had blood transfusion was 0.88 (0.00, 5.06) g/dL (Figure 14).

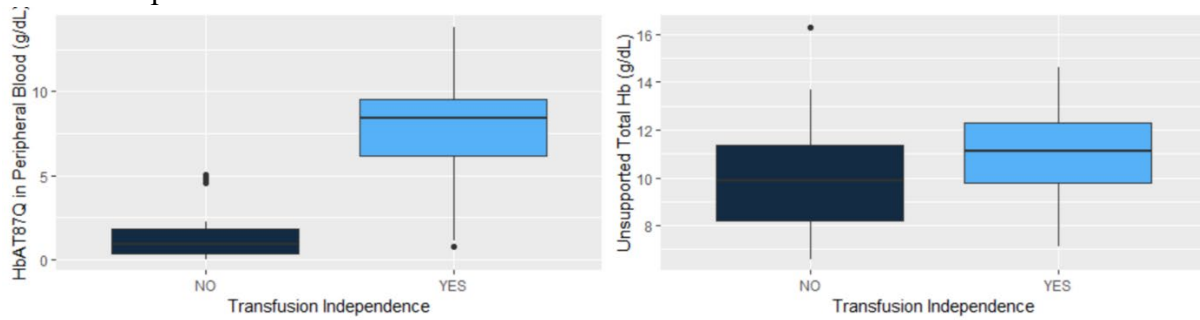
Univariate logistic analysis showed potential associations between PD parameters (HbA^{T87Q} and unsupported total Hb) and TI. Multivariate logistic analysis suggested positive association between the level of HbA^{T87Q} in peripheral blood and transfusion independence (no transfusion needs) (Table 12).

Table 12. Multivariate Correlations between PD Parameters and Transfusion Independence (no transfusion at the time point) (Phase 3 Studies)

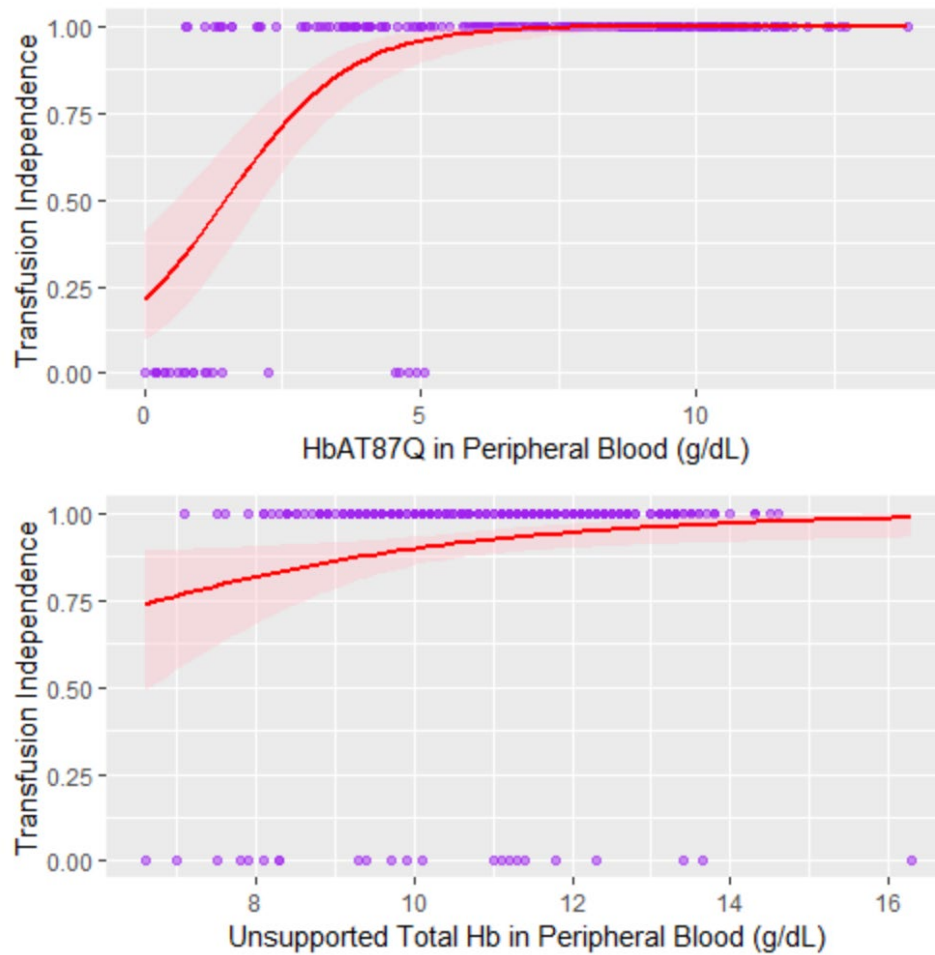
	Odds Ratio	95% CI	P value
Intercept	0.42	0.01 – 16.94	0.637
HbA ^{T87Q}	2.42	1.85 – 3.49	< 0.0001 ***
Unsupported Total Hb	0.96	0.66 – 1.34	0.804

Figure 14. PD Parameters and Transfusion Independence

a. Boxplot



b. Logistic Regression



6.7 Clinical Pharmacology Conclusions

ZYNTEGLO is a gene therapy consisting of autologous CD34+ cells containing hematopoietic stem cells (HSC) transduced with lentiviral vector (LVV) encoding β^{A-T87Q} -globin. After infusion of ZYNTEGLO, transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce red blood cells (RBCs) containing biologically active β^{A-T87Q} -globin that will combine with α -globin to produce functional Hb containing β^{A-T87Q} -globin (HbA^{T87Q}).

General Pharmacodynamics

- After infusion of beti-cel, beti-cel 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 levels in the Phase 3 studies was 1.293 (0.16, 4.52) c/dg (N=37). PB VCN levels generally remained stable as of the data cut off data of all studies. High inter-subject variability of PB VCN kinetic profiles was observed.
- HbA^{T87Q} generally increased steadily after administration of ZYNTEGLO, and stabilized by approximately Month 6 post-infusion. In the ongoing Phase 3 studies (Studies HGB-207 and HGB-212), subjects with TDT had a Month 6 median (min, max) HbA^{T87Q} of 8.74 (0.00, 12.01) g/dL (N = 35). HbA^{T87Q} remained durable with a median (min, max) of 8.80 (0.34, 12.43) g/dL at Month 24 (N = 30), demonstrating sustained expression of the β^{A-T87Q} protein derived from irreversible integration of the β^{A-T87Q} -globin gene into long-term HSCs.
- Intrinsic factors, such as genotype, age at baseline, race, sex, and weight at baseline did not impact the steady-state levels and time to steady-state for PB VCN and HbA^{T87Q}.
- Analysis of Hemoglobin (Hb) showed that HbA^{T87Q} was the major contributor to unsupported total Hb. The relative contributions of endogenous Hb may differ for each individual subjects.
- At Month 6 post-infusion of ZYNTEGLO, the median (min, max) unsupported total Hb levels were 11.55 (8.4, 13.3) g/dL (N=22) and 10.20 (8.5, 13.2) g/dL (N=15) in Phase 3 Study HGB-207 and HGB-212 respectively. The median unsupported total Hb levels were > 10 g/dL during the observation period of Phase 3 studies.

Dosing Characteristics and Responses

- 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 plateaued at higher levels of DP VCN after reaching approximately 80% to 90% DP %LVV+ cells. Positive correlation was also observed between DP %LVV+ cells and PB VCN levels at Month 6 post ZYNTEGLO infusion. Population PD analysis

also indicated that DP %LVV+ cells was the most important covariate impacting PB VCN levels.

- There was a significant correlative relationship between DP VCN and HbA^{T87Q} levels at Month 6 after infusion of ZYNTGLO: HbA^{T87Q} increased gradually with DP VCN at lower DP VCN values, but at higher DP VCN values, HbA^{T87Q} levels plateaued at approximately 8 to 10 g/dL. This observation indicates a feedback regulation of β -globin (or total Hb) levels within erythroid cells to maintain β -globin (or total Hb) levels below certain upper levels.
- There was a significant correlative linear relationship between DP %LVV+ cells and HbA^{T87Q} at Month 6: subjects received ZYNTGLO with higher DP %LVV+ cells had higher HbA^{T87Q} at Month 6, compared to subjects received ZYNTGLO with lower DP %LVV+ cells.
- No correlation between ZYNTGLO subpopulations and PB VCN and HbA^{T87Q} at post-infusion Month 6 and 24 were observed.
- There was no correlation observed between total CD34+ cell dose and HbA^{T87Q} in peripheral blood at either Month 6 or Month 24 for the *All TDT* pool, indicating that the lowest cell dose evaluated to date was adequate for effective reconstitution of HSCs in treated subjects.
- The targeted AUC range of busulfan used in clinical studies was considered adequate for myeloablation.

Pharmacodynamic Responses and Transfusion Independence

- PB VCN and HbA^{T87Q}: HbA^{T87Q} increased quickly with the increase of PB VCN at lower PB VCN levels, followed by an HbA^{T87Q} plateau at higher PB VCN levels. This observation suggests the regulation of β -globin levels within erythroid cells and selection of erythroid cells producing β^{A-T87Q} -globin during engraftment for balanced α -globin/ β -globin ratios.
- HbA^{T87Q} and transfusion independence (TI): Subjects with higher HbA^{T87Q} levels were less likely need blood transfusion. The median (min, max) HbA^{T87Q} at the time when no blood transfusion was needed was 8.44 (0.75, 13.85) g/dL and the median (min, max) HbA^{T87Q} at the time that subjects had blood transfusion was 0.88 (0.00, 5.06) g/dL.
- Unsupported total Hb at Month 6 and transfusion independence (TI): All of the 31 subjects who had ≥ 9 g/dL unsupported total Hb at Month 6 achieved TI. Statistically significant association was observed between unsupported total Hb level (≥ 9 g/dL) at Month 6 and TI.

7 APPENDIX - INDIVIDUAL STUDY

7.1 Study #1 – Study HGB-204

Study Completion: 21 February 2018.

<p>Title: A Phase 1/2, Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with 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: To evaluate the safety and efficacy of treatment with LentiGlobin BB305 Drug Product in subjects with TDT.</p>
<p>Methodology: Single-arm, multi-site, single dose study 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>Main Criteria for Inclusion: Subjects with TDT between 12 and 35 years of age (inclusive) at the time of consent/assent, with a transfusion history of ≥ 100 mL/kg/year of pRBC or ≥ 8 pRBC transfusions per year in each of the 2 years prior to enrollment.</p>
<p>Results: Disposition</p> <p>A total of 23 subjects signed the informed consent form (ICF) and were considered enrolled in the study; 19/23 subjects met the eligibility criteria. The first subject (Subject (b) (6)) had "inadequate stem cell mobilization", and was withdrawn from the study before myeloablative conditioning. Adequate numbers of stem cells were collected from all other eligible subjects (N = 18); they were treated with LentiGlobin BB305 Drug Product and completed the study.</p>
<p>Results: Pharmacodynamics</p> <ul style="list-style-type: none"> Eighteen subjects with TDT received LentiGlobin BB305 Drug Product intravenously as a single dose on Day 1, with a median (min, max) total cell dose of $8.10 (5.2, 18.1) \times 10^6$ CD34+ cells/kg, and with a median (min, max) DP VCN of $0.7 (0.3, 1.5)$ c/dg. All subjects who received LentiGlobin BB305 Drug Product (N = 18) had vector sequences in peripheral blood cells out to at least their Month 24 Visit. PB VCN values stabilized by approximately 6 months after LentiGlobin BB305 Drug Product infusion, with a median (min, max) of $0.356 (0.08, 0.95)$ c/dg at Month 6 and of $0.312 (0.06, 1.04)$ c/dg at Month 24, suggesting stable integration of the transgene into the genomes of long-term repopulating HSCs. All subjects who received LentiGlobin BB305 Drug Product (N = 18) produced HbA^{T87Q} out to at least their Month 24 Visit. HbA^{T87Q} increased steadily and generally stabilized by approximately 6 to 9 months after LentiGlobin BB305 Drug Product infusion with a median (min, max) of $4.561 (0.43, 8.89)$ g/dL at Month 6 and of $5.418 (0.47, 9.60)$ g/dL at Month 24, suggesting stable expression of the transgene in erythroid cells. No meaningful differences were observed for PB VCN and HbA^{T87Q} levels with respect to gender, age, race, and genotype, although the number of subjects analysed per subgroup was small. However, it was observed that HbA^{T87Q} kinetics for subjects with a non-β^0/β^0 genotype were generally more stable than those observed for subjects of β^0/β^0 genotype, most likely because the majority of subjects of β^0/β^0 genotype continued to receive regular pRBC transfusions after drug product infusion, which can cause suppression of erythropoiesis. Subjects who achieved TI status at any time during the study (N = 9) produced at Month 24 a median (min, max) total Hb of $10.00 (9.1, 13.7)$ g/dL, of which $6.407 (3.80, 9.60)$ g/dL was due to HbA^{T87Q}, $0.970 (0.08, 1.37)$ g/dL due to HbF, and $0.319 (0.19, 0.50)$ g/dL due to HbA₂. The 5 subjects with a β^E allele produced a median (min, max) of $3.277 (2.64, 4.78)$ g/dL of HbE. The 3 subjects with β^+ alleles produced 1.80 g/dL, 3.30 g/dL, or 6.44 g/dL of HbA. In these subjects, endogenous Hb levels generally stabilized at approximately the same time that transgenic HbA^{T87Q} levels stabilized.

- Stable ratios of α -globin/all β -like globins (as measured by RP-HPLC in the soluble fraction of hemolysates) of approximately 1 (range 1.07 to 1.34 at Month 24) were maintained in the absence of pRBC transfusions.
- Significant correlations were observed between DP VCN and PB VCN at Month 6 (N = 18, R = 0.6337, p = 0.0047), between DP VCN and HbA^{T87Q} at Month 6 (N = 18, R = 0.5904, p = 0.0099), and between HbA^{T87Q} and PB VCN both at Month 6 (N = 18, R = 0.8504, p < 0.0001). These results indicate that, within the range of DP VCN observed in this study (0.3 to 1.5 c/dg), higher DP VCN values generally resulted in higher PB VCN and HbA^{T87Q} levels in treated subjects.
- Significant correlations were observed between HbA^{T87Q} levels at Month 6 and HbA^{T87Q} levels at Month 24 (N = 18, R = 0.8305, p < 0.0001). These results indicate that HbA^{T87Q} levels at Month 6 are predictive of HbA^{T87Q} levels at Month 24.
- No significant correlation was observed between total cell dose and either the ratio of PB VCN M6/DP VCN (an indicator of the relative effectiveness of transduced HSC engraftment), or HbA^{T87Q} levels at Month 6, suggesting the cell doses received in this study ($\geq 5.2 \times 10^6$ CD34+ cells/kg) were adequate for effective reconstitution with transduced HSCs.
 - No significant correlation was observed between estimated average busulfan concentration and the ratio of PB VCN M6/DP VCN (an indicator of the relative effectiveness of transduced HSC engraftment), indicating that the estimated average busulfan dose range used during this study (3030 to 4714 $\mu\text{M} \cdot \text{min}$) is adequate for effective myeloablation of subjects.

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

The PD parameters, PB VCN and HbA^{T87Q} levels over time are summarized in Table 12

Table 13. Summary of PB VCN and HbA^{T87Q} in Peripheral Blood

Parameter Visit	PB VCN (c/dg)	HbA ^{T87Q} (g/dL)
Month 6		
N	18	18
Median	0.356	4.561
Min, Max	0.08, 0.95	0.43, 8.89
Month 24		
N	18	18
Median	0.312	5.418
Min, Max	0.06, 1.04	0.47, 9.60
Month 36		
N	18	18
Median	0.340	6.317
Min, Max	0.07, 1.82	0.38, 10.10
Month 48		
N	18	18
Median	0.333	6.178
Min, Max	0.07, 2.45	0.45, 9.43
Month 60		
N	14	14
Median	0.510	6.627
Min, Max	0.14, 1.80	2.84, 9.97

Year 6		
N	6	6
Median	0.432	6.578
Min, Max	0.21, 0.89	3.10, 9.49

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

7.2 Study #2 – Study HGB-212

Data Cut-Off Date: 09 March 2021.

Title: A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β -Thalassemia by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral β^{A-T87Q} -Globin Vector in Subjects ≤ 50 Years of Age				
Objectives: Evaluate the efficacy and safety of treatment with beti-cel in subjects in subjects ≤ 50 years of age with transfusion-dependent β -thalassemia (TDT) who have a β^0/β^0 , $\beta^0/IVS-I-110$, or $IVS-I-110/IVS-I-110$ genotype at the <i>HBB</i> gene.				
Methodology: Single-arm, multi-site, single dose study 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.				
Main Criteria for Inclusion: Subjects with TDT who have a β^0/β^0 , $\beta^0/IVS-I-110$, or $IVS-I-110/IVS-I-110$ genotype at the <i>HBB</i> gene; who are ≤ 50 years of age at the time of consent/assent; with a history of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment (all subjects), or be managed under standard thalassemia guidelines with ≥ 8 transfusions of pRBCs per year in the 2 years preceding enrollment (subjects ≥ 12 years).				
Results: Disposition and Dosing Nineteen subjects were mobilized with G-CSF and plerixafor (ITT population), 1 subject discontinued after 1 cycle of mobilization, and 18 subjects were conditioned with busulfan and administered beti-cel (TP). All TP subjects achieved successful neutrophil engraftment (SEP). Sixteen subjects have completed at least the Month 6 Visit, 15 subjects have completed the Month 12 Visit, and 11 subjects have completed the Month 24 visit.				
Summary of Key Dosing and Pharmacodynamic Parameters for Subjects with Available Month 6 Data				
Parameter	Statistic	β^0/β^0	Non- β^0/β^0	Overall
DP VCN (c/dg)	N	12	6	18
	Median	2.750	3.307	3.000
	Min, Max	1.20, 7.00	1.90, 4.70	1.20, 7.00
% LVV+ Cells in DP (%)	N	12	6	18
	Median	78.00	78.00	78.00
	Min, Max	34.0, 94.0	57.0, 85.0	34.0, 94.0
PB VCN at M6 (g/dL)	N	10	5	15
	Median	0.646	1.118	0.884
	Min, Max	0.16, 3.33	0.39, 2.97	0.16, 3.33
HbA ^{T87Q} at M6 (g/dL)	N	10	5	15
	Median	8.655	8.934	8.934
	Min, Max	0.00 ^a , 12.01	5.06, 10.33	0.00 ^a , 12.01
Unsupported Endogenous Hb at M6 (g/dL) ^b	N	9	5	14
	Median	1.42	2.10	1.46
	Min, Max	0.9, 6.3	1.2, 3.2	0.9, 6.3
Unsupported Total Hb at M6 (g/dL) ^b	N	9	6	15
	Median	10.10	10.25	10.20

	Min, Max	8.8, 13.2	8.5, 12.2	8.5, 13.2
Abbrev: DP, drug product; Hb, hemoglobin; HbA ^{T87Q} , hemoglobin A containing β^{A-T87Q} -globin; M6, Month 6; NA, not applicable; VCN, vector copy number				
^a This value was recorded as zero for Subject (b) (6) ; although this subject did have consistently low HbA ^{T87Q} values after Month 6 visit, all other values were above zero.				
^b Only includes measurements assessed without any pRBC transfusions in at least the prior 60 days.				
<ul style="list-style-type: none"> Eighteen subjects received beti-cel intravenously on Day 1, with a median (min, max) total cell dose of $10.75 (5.9, 42.1) \times 10^6$ CD34+ cells/kg. Median (min, max) DP VCN was 3.00 (1.20, 7.00) c/dg and median (min, max) %LVV+ Cells in drug product was 78.00% (34.0%, 94.0%). Estimated daily average area under the curve (AUC) of the busulfan dose ranged from 3605 to 9086 $\mu\text{M} \cdot \text{min}$. All treated subjects (N = 18) have detectable vector sequences in the peripheral blood through last follow-up. The PB VCN was variable between subjects, but for each subject was relatively stable from first assessment at Month 2 through Month 24, suggesting persistence of transduced HSCs and their ongoing differentiation into transgene-containing progeny in peripheral blood. Median (min, max) PB VCN at Month 6 was 0.884 (0.16, 3.33) c/dg c/dg (N = 15). For the 15 subjects with Month 6 HbA^{T87Q} data available, median (min, max) HbA^{T87Q} was 8.934 (0.00, 12.01) g/dL (see footnote in table above for note on zero value). HbA^{T87Q} levels generally stabilized by approximately 6 months after drug product infusion, indicating sustained expression of the transgene. The 15 subjects with Hb fraction data at Month 6 had median (min, max) total Hb of 10.20 (8.5, 13.2) g/dL (N = 15) within this period, with the majority of subjects able to maintain total Hb levels ≥ 9 g/dL, which is sufficient to meet the criteria required for transfusion independence. HbA^{T87Q} was the primary contributor to total Hb levels, and 8 of 15 subjects had $\text{HbA}^{\text{T87Q}} \geq 9$ g/dL. <p>At Month 6, in the absence of pRBC transfusions, subjects maintained a normal or near-normal median (min, max) α-globin/β-like-globin ratio of 1.126 (0.95, 1.28) (N = 15).</p>				

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

7.3 Study #3 – Study LTF-303

Data Cut-off Date: 09 March 2021.

Title: Long-term Follow-up of Subjects with Hemoglobinopathies Treated with Ex Vivo Gene Therapy Using Autologous Hematopoietic Stem Cells Transduced with a Lentiviral Vector
Objectives: Monitor for long-term safety and efficacy of the gene therapy drug product used in a bluebird bio-sponsored clinical studies (i.e., the “parent studies”) in treated subjects with hemoglobinopathies.
Methodology: Single arm, multi-center, non-interventional long-term follow-up study. After monitoring of a subject in the parent study was completed (where the parent study includes approximately 2 years of follow-up after drug product infusion), subjects were eligible to enroll in Study LTF-303. During Study LTF-303, subjects were followed every 6 months from 2 years through 5 years post-drug product infusion and then annually from 5 years through 15 years post-drug production infusion for a total of 13 years of follow-up in Study LTF-303.
Main Criteria for Inclusion: Subjects treated with drug product for therapy of a hemoglobinopathy in a bluebird bio-sponsored clinical study who have provided written informed consent, or written informed consent from their parent[s]/legal guardian[s], as applicable, and who are able to comply with study requirements.

Results: Disposition

Fifty-one subjects with TDT treated with beti-cel (18 from Study HGB-204, 4 from Study HGB-205, 19 from Study HGB-207, and 10 from Study HGB-212) consented to enroll in Study LTF-303, and all of these subjects continue to participate in Study LTF-303 as of the interim database lock for this CSR, with a median (min, max) follow-up time of 44.22 (22.9, 86.5) months post-drug product infusion.

No subjects have yet completed Study LTF-303.

Results: Demography

Of the 51 enrolled beti-cel treated subjects with TDT, 28 were female (54.9%) and 23 were male (45.1%). Median (min, max) age at consent or assent in parent study was 19.0 (7, 35) years; 30 (58.8%) subjects were ≥ 18 years of age, 13 (25.5%) subjects were ≥ 12 and < 18 years of age, and 8 (15.7%) subjects were < 12 years of age. Twenty-seven (52.9%) subjects identified as Asian and 22 (43.1%) subjects identified as White. Thirty-seven (72.5%) subjects were of non- β^0/β^0 genotype and 14 (27.5%) subjects were of β^0/β^0 genotype.

Results: Pharmacodynamics**For Beti-cel-treated Subjects with TDT (N = 51):*****Vector Copy Number***

- PB VCN generally remained stable during Study LTF-303 in all subjects, including 8 subjects who completed their Year 6 Visit and 1 subject who completed their Year 7 Visit, confirming long-term stable reconstitution of peripheral nucleated cells from lentiviral vector (LVV)-modified stem cells.
- Median (min, max) PB VCN was observed to be higher for subjects treated in Phase 3 studies (1.120 [0.11, 4.55] c/dg [N = 29]) compared to those treated in Phase 1/2 studies (0.404 [0.07, 3.86] c/dg [N = 22]) at last follow-up due to optimization of the manufacturing conditions during the clinical development program.

HbA^{T87Q} Expression

- HbAT87Q generally remained stable in all subjects during Study LTF-303, demonstrating long-term stable production of transgenic HbAT87Q.
- Median (min, max) HbAT87Q was observed to be higher for subjects treated in Phase 3 studies (9.386 [0.23, 12.72] g/dL [N = 29]) compared to those treated in Phase 1/2 studies (6.627 [0.35, 10.71] g/dL [N = 22]) at last follow-up due to optimization of the manufacturing conditions during the clinical development program.
- 8 subjects had HbAT87Q levels with a median (min, max) of 7.423 (3.10, 9.49) g/dL at Year 6 and the subject with longest follow-up had a level of 10.714 g/dL at Year 7.
- Endogenous Hb production remained stable during Study LTF-303.

Ratio of α -Globin to β -Like-Globin

- Stable ratios close to 1.0 of α -globin to all β -like-globins were maintained in subjects who have achieved TI, with a median (min, max) ratio of 1.128 (0.86, 1.34) and 1.084 (0.96, 1.48) at Month 24 (N = 40) and Year 6 (N = 7), respectively.

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

7.4 Study #4 – Population Pharmacodynamic Analysis (Study # 261903)

The Applicant developed population PD models to describe the PD profiles of PB VCN and HbA^{T87Q} following a one time IV dose of beti-cel (LentiGlobin BB305) drug product in patients with transfusion-dependent β -thalassemia (TDT). The PD models 1) quantified population parameters, including typical parameter values and random inter-individual and residual variability terms; and 2) identified and quantified covariate effects with describe variability in PB VCN and HbA^{T87Q}, especially the influence of age and weight, to understand the dose-exposure-response relationship with pediatric patients, and 3) derive individual model predicted

steady-state levels and individual model predicted time to approach steady-state for PB VCN and HbA^{T87Q}.

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

7.4.1 Data Source

Population PD models were built using data from Studies HGB-204, HGB-207, HGB-212. Data from HGB-205 were excluded due to lack of DP %LVV+ cells data. A total of 566 and 560 observations were included for building PD models of PB VCN and HbA^{T87Q} respectively (Table 14).

Table 14. Summary of Data Available for Population PD Model Building

Study	Study Description	TDT Subjects	DP VCN c/dg	DP LVV+ Cells %	Cell Dose CD34+ cells/kg
HGB-204	A Phase 1/2 open label study evaluating the safety and efficacy of LentiGlobin BB305 Drug Product in subjects with β -thalassemia major.	10 non- β^0/β^0 8 β^0/β^0	0.3 – 1.50	17 – 58	5.2 – 18.1 x 10 ⁶
HGB-207	A Phase 3 single arm study evaluating the safety and efficacy of LentiGlobin BB305 Drug Product in subjects with TDT, who do not have β^0/β^0 genotype in subjects \leq 50 years of age.	23 non- β^0/β^0	1.9 – 5.6	34 – 90	5.0 - 19.9 x 10 ⁶
HGB-212	A Phase 3 single arm study evaluating the safety and efficacy of LentiGlobin BB305 Drug Product in subjects with TDT, in subjects \leq 50 years of age.	6 non- β^0/β^0 12 β^0/β^0	1.2 – 7.0	34 – 94	5.0 – 42.1 x 10 ⁶

If a subject had multiple lots of drug product, the Drug Product vector copy number (DP VCN) and percent lentiviral vector positive cells in Drug Product (DP %LVV+ Cells) were measured per lot then the fractions of (drug product dose per lot/total drug product dose of all lots) were used as weight to calculate a weighted average per subject.

Source: Applicant. Module 5, section 5.3.4.2. Population Pharmacodynamic Analysis Report

7.4.2 Methods

7.4.2.1 Population PD Model for PB VCN

Base Model

After administration of beti-cel, the median PB VCN levels increased over a period of time before reaching a plateau. The Applicant developed two base models:

- Linear-plateau model

$$PB \ VCN(t) = \begin{cases} \text{Baseline} + K_{TA} \cdot t & \text{for } t < T_{\max, PB \ VCN} \\ VCN_{MAX} & \text{for } t \geq T_{\max, PB \ VCN} \end{cases}$$

Baseline: baseline PB VCN; K_{TA} : rate of appearance of cells containing integrated transgene; t: time; $T_{\max, PBVCN}$: the time of the maximum PB VCN; VCN_{MAX} : model estimated maximum PB VCN.

- Exponential asymptotic growth model

$$PB \ VCN(t) = VCN_{MAX} * (1 - e^{(-K_{TA} \cdot t)})$$

VCN_{MAX} : model estimated maximum PB VCN; K_{TA} : rate of appearance of cells containing integrated transgene; t: time.

Graphical diagnostics and statistical evaluation using the change in objective function value (ΔOBJ) techniques were utilized to assess how well the model fit the data.

Covariates

Following covariates were tested: DP VCN, transduction index in drug product, DP %LVV+ Cells, manufacturing process, age at baseline, age category, weight at baseline, weight category, average busulfan AUC, genotype, sex and race.

7.4.2.2 Population PD Model for HbA^{T87Q}

Base Model

Three base models were developed to describe PD profiles of HbA^{T87Q}:

- Linear-plateau model

$$HbA^{T87Q}(t) = \begin{cases} \text{Baseline} + K_{HB} \cdot t & \text{for } t < T_{\max, HbA^{T87Q}} \\ Hb_{MAX} & \text{for } t \geq T_{\max, HbA^{T87Q}} \end{cases}$$

Baseline: baseline HbA^{T87Q} value (defined as zero), K_{HB} is the rate of HbA^{T87Q} production, t: time; $T_{\max, HbAT87Q}$: the time of the maximum HbA^{T87Q}, Hb_{MAX} : the model estimated maximum HbA^{T87Q}.

- Exponential asymptotic growth model

$$HbA^{T87Q}(t) = Hb_{MAX} \cdot (1 - e^{-(K_{HB} \cdot t)^\gamma})$$

Hb_{MAX} : model estimated maximum HbA^{T87Q}; K_{HB} : rate of HbA^{T87Q} production; t: time; and γ is the shape parameter.

- Emax model

$$\text{HbA}^{\text{T87Q}}(t) = \text{Hb}_{\text{MAX}} \cdot \frac{t^\gamma}{\text{ET}_{50, \text{HbA}^{\text{T87Q}}}^\gamma + t^\gamma}$$

Hb_{MAX}: model estimated maximum HbA^{T87Q}; t: time; ET_{50, HbA^{T87Q}} the estimated time for HbA^{T87Q} to reach 50% of its maximum value, the γ is the shape parameter that controls the steepness of the curve.

Covariates

Following covariates were examined: DP VCN, transduction index in drug product, DP %LVV+ Cells, manufacturing process, age at baseline, age category, weight at baseline, weight category, average busulfan AUC, genotype, sex, race, maximum PB VCN, rate of transgene appearance, total transfusion volume (pre-DP infusion), and transfusion volume (post-DP infusion).

7.4.3 Results

7.4.3.1 Final Population PD Model for PB VCN

The best model to fit the available data was the exponential asymptotic growth model.

$$\text{PB VCN}(t) = \text{VCN}_{\text{MAX}} * (1 - e^{(-K_{\text{TA}} \cdot t)})$$

PB VCN was modeled in terms of VCN_{MAX} and K_{TA}, with BSV terms incorporated on both parameters. A proportional error model was used to describe the residual unexplained variability (RUV).

As shown in Table 15, DP %LVV+ Cells was included as a covariate on VCN_{MAX} and manufacturing process was included as a covariate on K_{TA}.

Table 15. Parameter Estimates for the Final PB VCN Model

Parameter Name	Estimated Value (%RSE)	Bootstrap Median (95% CI)
Estimate of VCN _{pop} (c/dg)	0.831 (14.5)	0.825 (0.597 – 1.07)
Slope for DPLVVP+ Cells ≤ 75% (/%)	0.0139 (11.6)	0.0139 (0.0081 – 0.0159)
Slope for DPLVVP+ Cells >75% (/%)	0.171 (27.8)	0.169 (0.010 – 0.308)
Estimate of K _{TA} (/month)	5.87 (23.3)	6.59 (3.23 – 12.7)
Fractional decrease in K _{TA} for Process 1 (unitless)	-0.721 (5.3)	-0.751 (-0.881 – -0.0947)
Between Subject Variability for VCN _{MAX} (%CV)	56.3 (11.2)	54.1 (42.5 – 67.1)
Between Subject Variability for K _{TA} (%CV)	124 (24.5)	120 (56.4 – 201)
Residual Unexplained Variability (Proportional, %CV)	20.3 (11.1)	20.1 (16.6 – 24.8)

RSE = relative standard error, %CV = % coefficient of variation.

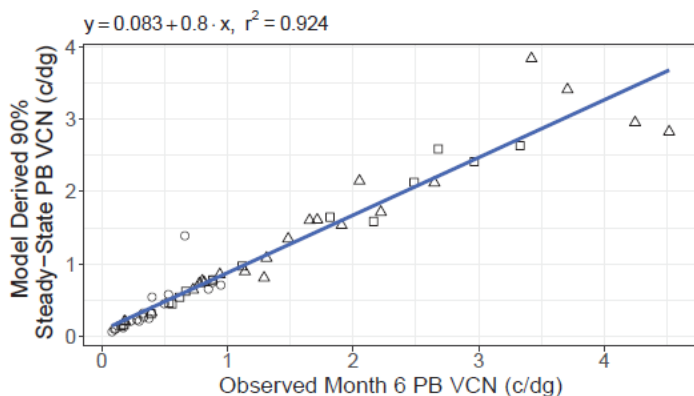
^a 95% CI is reported from the 2.5th and 97.5th percentiles of the estimated values from 940 bootstrap datasets.

Source: Applicant. Module 5, section 5.3.4.2. Population Pharmacodynamic Analysis Report

Age, weight, genotype, sex and race were not significant covariates and did not correlate steady-state maximum PB VCN or the time to 90% steady-state maximum.

Figure 15 shows the comparison between observed Month 6 PB VCN and model derived steady-state PB VCN.

Figure 15. Comparison of Observed Month 6 PB VCN and Model Derived Steady-State PB VCN



Source: Applicant. Module 5, section 5.3.4.2. Population Pharmacodynamic Analysis Report

7.4.3.2 Final Population PD Model for HbA^{T87Q}

The best model to fit the available data was the maximum effect (Emax) model with a Hill slope.

$$\text{HbA}^{\text{T87Q}}(t) = \text{Maximum HbA}^{\text{T87Q}} \cdot \frac{t^\gamma}{ET_{50, \text{HbA}^{\text{T87Q}}}^\gamma + t^\gamma}$$

HbAT87Q was modeled in terms of maximum HbA^{T87Q} (Hb_{MAX}) and time to 50% maximal HbA^{T87Q} (ET_{50,HbA^{T87Q}}), with BSV applied to both parameters, and a Hill slope (γ). A proportional error model was used to describe the residual unexplained variability (RUV).

As shown in Table 16, VCN_{MAX} derived from PB VCN model was included on Hb_{MAX}. Manufacturing process was included as a covariate on Hillslope (γ).

Table 16. Parameter Estimates for the Final HbA^{T87Q} Model

Parameter Name	Estimated Value (%RSE)
Estimate of Hb _{pop} (g/dL)	9.4 (6.4)

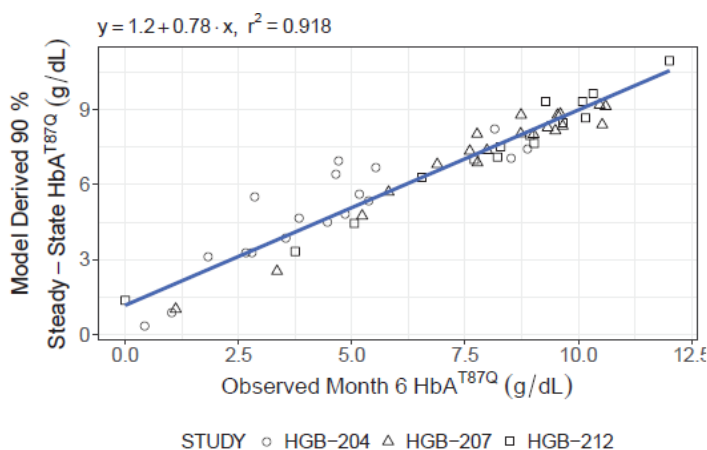
Estimate of VCN _{MAX,50} (c/dg)	0.296 (18.7)
Hill Slope for VCN Covariate (unitless)	1.79 (19.8)
Estimate of ET ₅₀ (/month)	2.42 (4.2)
Hill Slope (unitless)	3.85 (7.4)
Decrease in Hill Slope for Process 1 (fraction)	-0.274 (27.8)
Between Subject Variability for Hb _{MAX} (%CV)	29.6 (21.1)
Between Subject Variability for ET ₅₀ (%CV)	26.3 (13.5)
Residual Unexplained Variability (Proportional, %CV)	19.7 (12.8)

Source: Applicant. Module 5, section 5.3.4.2. Population Pharmacodynamic Analysis Report

Age, weight, genotype, sex and race were not significant covariates in the HbA^{T87Q} model ($p > 0.01$) and did not correlate with steady-state maximum HbA^{T87Q} or the time to 90% steady-state maximum.

Comparison between observed Month 6 HbA^{T87Q} and model derived steady-state HbA^{T87Q} is shown in Figure 16.

Figure 16. Comparison of Observed Month 6 HbA^{T87Q} and Model Derived Steady-State HbA^{T87Q}



Open symbols = individual data, shapes = study, blue line = linear regression, the regression model and coefficient of determination are shown above each panel.

Source: Applicant. Module 5, section 5.3.4.2. Population Pharmacodynamic Analysis Report

Reviewer's Comments:

The Applicant's models for PB VCN and HbAT87Q are generally acceptable to assess covariates impact. Due to limited experiences with beti-cel, using modeling for prediction (for both PD and clinical efficacy responses) is considered as exploratory.