

Clinical Pharmacology BLA Review

Division of Clinical Evaluation General Medicine
Office of Clinical Evaluation
Office of Therapeutic Products

BLA	125785/0
Product	CASGEVY (exagamglogene autotemcel, exa-cel) Suspension for Intravenous Infusion
Sponsor	Vertex Pharmaceuticals, Inc.
Indication	Treatment of transfusion dependent β -thalassemia (TDT)
Date Received	March 31, 2023
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1 EXECUTIVE SUMMARY

Vertex Pharmaceuticals, Inc. seeks approval of its BLA for CASGEVY (exagamglogene autotemcel, exa-cel, CTX001 or VX-290) for treatment of transfusion dependent β -thalassemia (TDT). CASGEVY is a cellular gene therapy consisting of autologous CD34⁺ human hematopoietic stem and progenitor cells (hHSPCs) modified by CRISPR-Cas9-mediated gene editing of the erythroid lineage-specific enhancer region of the BCL11A gene. CASGEVY is a cell suspension for a one-time single dose intravenous infusion. The proposed minimum dose of CASGEVY is 3.0×10^6 CD34⁺ cells/kg.

The clinical pharmacology evaluation of this biologics license application (BLA) includes one ongoing Phase 1/2/3 study (Study CTX001-111), one long-term follow-up study (Study CTX001-131), and a population pharmacodynamic (PD) study (Study T067). Based on the nature of CASGEVY, conventional studies on pharmacokinetics, absorption, distribution, metabolism, and elimination cannot be used to monitor the presence of the drug product and are not applicable. Clinical pharmacology review focuses on the pharmacodynamic responses after administration of CASGEVY.

After CASGEVY infusion, the edited CD34⁺ cells engraft in the bone marrow and differentiate to erythroid lineage cells with reduced BCL11A expression. Reduced BCL11A expression results in an increase in γ globin expression and HbF protein production in erythroid cells. In subjects who received CASGEVY, the mean proportion of alleles with the intended genetic modification in CD34⁺ cells of bone marrow remained stable (>75%) at Month 6 onward. Allelic editing in the

peripheral blood was detectable at Month 1, and remained stable (> 62%) from Month 2 onwards. Increases in mean (SD) total hemoglobin (total Hb) and fetal hemoglobin (HbF) levels were observed as early as Month 3 after CASGEVY infusion and continued to increase to 12.2 (SD: 2.0) g/dL and 10.9 (SD: 2.8) g/dL respectively at Month 6. After Month 6, levels of total Hb and HbF were maintained thereafter, with HbF comprising $\geq 88\%$ of total Hb. Consistent with the increase in HbF levels, the mean (SD) proportion of circulating erythrocytes expressing HbF (F-cells) at Month 3 was 73.8% (SD: 19.7%) and continued to increase over time to 95.9% (SD: 15.2%) at Month 6, with levels remaining stable thereafter, indicating sustained pan-cellular expression of HbF. No dose-response relationship was identified for clinical efficacy (transfusion independence).

The proposed dosing regimen of CASGEVY 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

β -thalassemia (TDT) is caused by a spectrum of mutations that result in reduced or absent production of adult hemoglobin (HbA). The degree of impaired HbA production, resulting from the extent of incomplete (β^+) or absent (β^0) β -globin expression, determines the severity of β -thalassemia. Reduction in β -globin production results in an accumulation of excess, α -globin in erythroblasts. The clinical implications of this α -globin/ β -globin imbalance are hemolysis leading to a lack of sufficient erythrocytes and Hb (anemia) to effectively transport oxygen throughout the body.

CASGEVY (exagamglogene autotemcel, exa-cel, CTX001 or VX-290) is a cellular product consisting of autologous CD34⁺ hematopoietic stem and progenitor cells (HSPCs) modified by CRISPR-Cas9-mediated gene editing of the erythroid enhancer region of the *BCL11A* gene. Cas9 is a nuclease enzyme that uses CRISPR single guide RNA (sgRNA) sequences to cleave a specific genomic locus that is complementary to the CRISPR sgRNA. The goal of CASGEVY treatment is to reactivate fetal hemoglobin (HbF) production to levels known to eliminate disease complications, consistent with individuals with β -thalassemia who co-inherit hereditary persistence of fetal hemoglobin (HPFH), in whom high levels of HbF expression continue throughout life.

After CASGEVY infusion, the edited CD34⁺ cells engraft in the bone marrow and differentiate to erythroid lineage cells with reduced BCL11A expression. Reduced BCL11A expression results in an increase in γ -globin expression and HbF protein production in erythroid cells. In patients with

transfusion-dependent β -thalassemia, γ -globin production improves the α -globin to non- α -globin imbalance thereby reducing ineffective erythropoiesis and hemolysis and increasing total hemoglobin levels, addressing the underlying cause of disease and eliminating the dependence on regular red blood cell (RBC) transfusions.

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

This clinical pharmacology section of this application includes one ongoing Phase 1/2/3 study (Study CTX001-111), one long-term follow-up study (Study CTX001-131), and a population pharmacodynamic (PD) study (Study T067).

3 SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY FINDINGS

Key clinical pharmacology findings are summarized below:

Pharmacokinetic and Pharmacodynamics of Conditioning Regimen – Busulfan

Busulfan Dose Regimen: a total of 53 subjects underwent busulfan conditioning. The mean (SD) administered busulfan dose was 3.54 (0.481) mg/kg/day for q6h regimen group (n=23) and 3.88 (0.742) dose of busulfan mg/kg/day for the qd regimen group (n=30).

Busulfan Exposure: with the q6h regimen, 4 subjects had an exposure below the target range and 2 subjects had exposures above the target range. With the qd regimen, 6 subjects had an exposure below the target range and 8 subjects had exposures above the target range. Intrinsic factors (age, sex, race, genotype, and body weight) did not show significant impact on busulfan exposure.

Busulfan Dose Regimen/Exposure and Engraftment: no evident clinically relevant relationship was observed between individual subject time to neutrophil or platelet engraftment and observed busulfan cumulative area under the curve (cAUC) or busulfan dose regimen (q6h or qd).

Busulfan Exposure and Clinical Efficacy (TI12): no evident clinically relevant relationship was observed between busulfan exposure and primary clinical efficacy endpoint (TI12) was observed.

Pharmacodynamic Responses After Administration of CASGEVY

Persistence of Edited Cells: the mean proportion of alleles with the intended genetic modification in CD34+ cells of bone marrow remained stable (>75%) at Month 6 onward. Allelic editing in the peripheral blood was detectable within 1 month and remained stable (> 62%) from Month 2 onward.

Total Hemoglobin (total Hb) and Fetal Hemoglobin (HbF): increases in mean (SD) total Hb and HbF levels were observed as early as Month 3 after CASGEVY infusion and continued to increase to 12.2 (2.0) g/dL and 10.9 (2.8) g/dL respectively at Month 6. After Month 6, levels of total Hb and HbF were maintained thereafter, with HbF comprising $\geq 88\%$ of total Hb.

Proportion of Circulating Erythrocytes Expressing HbF (F-Cells): consistent with the increase in HbF levels, the mean (SD) proportion of circulating erythrocytes expressing HbF (F-cells) at Month 3 was 73.8% (19.7%) and continued to increase over time to 95.9% (15.2%) at Month 6, with levels remaining stable thereafter, indicating sustained pan-cellular expression of HbF.

Durability of Pharmacodynamic Responses: the durability of pharmacodynamic responses was observed up to Month 42 post infusion of CASGEVY. Durability of PD responses was also supported by correlation analysis of PD biomarkers at different visits and population pharmacodynamic modeling.

Subgroup Analysis: no significant impact of intrinsic factors (age, sex, race, and genotype) and extrinsic factor (busulfan dose and exposure) on pharmacodynamic responses were observed in subjects with TDT.

Dose-Responses of CASGEVY

Dosing Characteristics and Pharmacodynamic Responses: higher CASGEVY dose level (body weight-based) was potentially associated with higher proportion of allelic editing in bone marrow at Month 6.

Dose-Response Relationship for Clinical Efficacy: no dose-response relationship was identified for clinical efficacy (transfusion independence, TI12).

Relationships between Pharmacodynamic Biomarkers

Following positive correlations (Pearson correlation coefficient > 0.7 and $p < 0.05$) were observed between pharmacodynamic biomarkers at Month 6 post administration of CASGEVY:

- Allelic editing in bone marrow vs. allelic editing in peripheral blood.
- Total Hb level vs. HbF level
- HbF vs. HbF%
- HbF vs. proportion of F-Cells (%)
- HbF% vs. proportion of F-Cells (%)

Pharmacodynamic Biomarkers and Clinical Efficacy Outcome

Total Hemoglobin Levels and Transfusion Independence (TI12): all 30 subjects who had unsupported total Hb at Month 6 of ≥ 9 g/dL achieved TI12. Among 5 subjects who had unsupported total Hb at Month 6 of < 9 g/dL, 2 of them achieved TI12.

4 LABELING COMMENTS

The clinical pharmacology reviewer has reviewed the package insert for BLA 125785/0 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 move the pharmacodynamic biomarkers information presented in *Section 14 Clinical Studies* to *Section 12.2 Pharmacodynamics*.

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

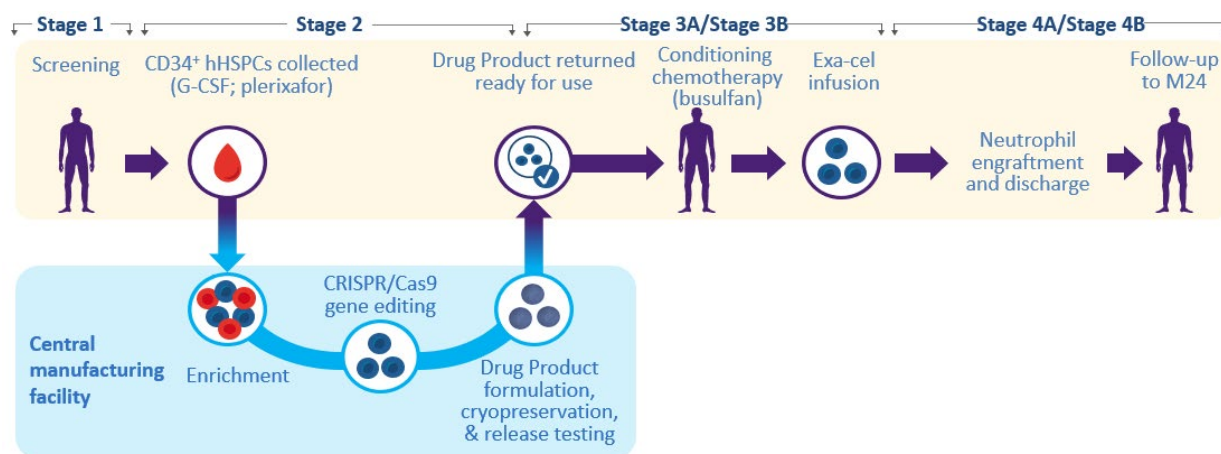
The clinical pharmacology review team's recommendation for approval of CASGEVY is based on review of data from the one ongoing Phase 1/2/3 study (Study CTX001-111), and supportive data from one long-term follow-up study (Study CTX001-131), and a population pharmacodynamic (PD) study (Study T067).

Study Design

As shown in Figure 1, Study CTX001-111 was conducted in 4 stages:

- Stage 1 was a screening and pre-mobilization period to determine eligibility.
- Stage 2 included mobilization (granulocyte colony-stimulating factor [G-CSF] and plerixafor), CD34⁺ stem cell collection, and exa-cel manufacture and disposition.
- Stage 3 included myeloablative conditioning (Stage 3A) and exa-cel infusion (Stage 3B).
- Stage 4 included follow-up, through neutrophil engraftment (Stage 4A) and up to 24 months after exa-cel infusion (Stage 4B).

Figure 1. Study CTX001-111 Study Design



Source: Applicant. Study CTX001-111 CSR.

6.2 Dosing Characteristics

6.2.1 Busulfan

Prior to infusion of CASGEVY, busulfan was used as a single agent for myeloablative bone marrow conditioning in Study CTX001-111.

6.2.1.1 Busulfan Dosing Regimen

Busulfan administered intravenously (IV) daily at a starting dose of 3.2 mg/kg/day for 4 consecutive days (based on body weight measured within 3 to 7 days before the first day of busulfan administration). Target busulfan cumulative exposure (cAUC) for each dosing regimen remained the same across all age groups. The target cAUC was 74 mg*h/L (target range: 59 to 89) for q6h regimen (1125 µM*min [range: 900 to 1350]) and 82 mg*h/L (target range: 74 to 90) for qd regimen (5000 µM*min [range: 4500 to 5500]).

As shown in Table 1, a total of 53 subjects underwent busulfan conditioning. The mean (SD) administered busulfan dose was 3.54 (0.481) mg/kg/day for q6h regimen group (n=23) and 3.88 (0.742) dose of busulfan mg/kg/day for qd regimen group (n=30).

Table 1. Mean Busulfan Dose Administered and Observed cAUC by Dosing Regimen

Dosing Regimen	Statistic	Busulfan Dose (mg/kg/day)	Busulfan cAUC (mg·h/L)
All	N	53	53
	Mean (SD)	3.73 (0.659)	77.9 (15.0)
	Median (min, max)	3.73 (2.65, 6.21)	76.4 (47.9, 126)
q6h	n	23	23
	Mean (SD)	3.54 (0.481)	68.4 (12.4)
	Median (min, max)	3.56 (2.65, 4.54)	67.2 (47.9, 103)
qd	n	30	30
	Mean (SD)	3.88 (0.742)	85.2 (12.7)
	Median (min, max)	3.76 (2.72, 6.21)	85.1 (61.9, 126)

Source: Applicant. Study CTX001-111 CSR.

With the q6h regimen, 4 subjects had an exposure below the target range and 2 subjects had exposures above the target range. With the qd regimen, 6 subjects had an exposure below the target range and 8 subjects had exposures above the target range (Table 2). In subgroup analyses, no clinically relevant effects of age at screening (≥ 12 and < 18 years of age versus ≥ 18 and ≤ 35 years of age), sex, race, or genotype (β^0/β^0 -like versus non- β^0/β^0 -like) on busulfan cAUC were observed. No clinically relevant effect of body weight was observed.

Table 2. Percentage of Subjects Achieving Target Cumulative Busulfan Exposure (cAUC) by Dosing Regimen

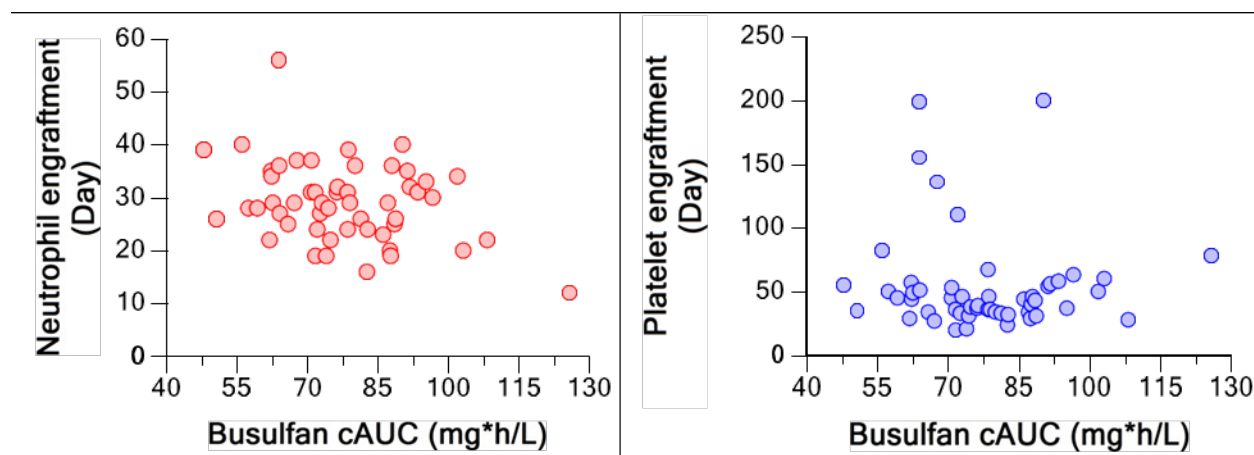
Dosing Regimen	cAUC Below Target Range	n (%) for Subjects With	
		cAUC Within Target Range	cAUC Above Target Range
All (N = 53)	10 (19%)	33 (62%)	10 (19%)
q6h (N = 23)	4 (17%)	17 (74%)	2 (9%)
qd (N = 30)	6 (20%)	16 (53%)	8 (27%)

Source: Applicant. Study CTX001-111 CSR.

6.2.1.2 Busulfan Exposure and Engraftment

The relationships between busulfan exposure and engraftment (neutrophil and platelet engraftment) were evaluated. No evident clinically relevant relationship was observed between individual subject time to neutrophil or platelet engraftment and observed busulfan cAUC or busulfan dose regimen (q6h or qd) (Figure 2).

Figure 2. Individual Subject Time to Neutrophil Engraftment (Left) or Platelet Engraftment (Right) After CASGEVY Infusion Versus Observed Busulfan cAUC



Source: Applicant. Study CTX001-111 CSR.

6.2.1.3 Busulfan Exposure Versus Pharmacodynamic Responses and Transfusion Independence (TI12)

No evident association between busulfan exposure and pharmacodynamic responses was observed.

In the primary efficacy set (N=35), three subjects did not achieve the primary efficacy endpoint (TI12). One subject had cAUC of busulfan within the target range and two subjects had cAUC higher than the target range. No significant impact of busulfan exposure on TI12 was observed.

6.2.2 CASGEVY (exa-cel)

The minimum dose of CASGEVY to be administered to subjects with TDT was primarily based on accepted safe clinical practices regarding the dose of CD34+ cells required to achieve durable long-term hematopoietic reconstitution after autologous transplantation. In clinical practice, autologous transplantation for various indications typically employ a minimum of 2×10^6 to 2.5×10^6 CD34+ cells/kg to support engraftment. The protocol defined dose of CASGEVY was a minimum of 3.0×10^6 CD34+ cells/kg and a maximum of 20×10^6 CD34+ cells/kg in Study

CTX001-111.

In Study CTX001-111, a total of 52 subjects with TDT received CASGEVY. The median (range) dose of CASGEVY was 7.5 (range: 3.0 to 19.7) x 10⁶ CD34⁺ cells/kg.

The proportion of alleles with the intended genetic modification in CASGEVY drug product is summarized in Table 3. The mean (SD) proportion of alleles with the intended genetic modification in CASGEVY drug product were 86.41 (9.90%) and 88.04% (8.53%) in primary efficacy set (PES) and full analysis set (FAS), respectively.

Table 3. Summary of the Proportion of Alleles with the Intended Genetic Modification in the CASGEVY Drug Product

Category	PES N = 35	FAS ^a N = 52
Exa-cel product editing (%)		
n	35	52
Mean (SD)	86.41 (9.90)	88.04 (8.53)
Median	90.82	91.18
Min, max	58.43, 94.65	58.43, 95.44

PES: Primary Efficacy Set FAS: Full Analysis Set

Source: Applicant. Study CTX001-111 CSR.

The relationships between CASGEVY drug product dosing characteristics and pharmacodynamic and clinical responses are discussed in Section 6.5.

6.3 General Pharmacology

After CASGEVY infusion, the edited CD34⁺ cells engraft in the bone marrow and differentiate to erythroid lineage cells with reduced BCL11A expression. Reduced BCL11A expression results in an increase in γ -globin expression and HbF protein production in erythroid cells. In patients with transfusion-dependent β -thalassemia, γ -globin production improves the α -globin to non- α -globin imbalance thereby reducing ineffective erythropoiesis and hemolysis and increasing total hemoglobin levels, addressing the underlying cause of disease and eliminating the dependence on regular red blood cell (RBC) transfusions.

Based on the nature of CASGEVY (exa-cel), conventional studies on pharmacokinetics, absorption, distribution, metabolism, and elimination cannot be used to monitor the presence of the drug product and are not applicable. To evaluate the delivery and persistence of CASGEVY (exa-cel), pharmacodynamic (PD) parameters were measured to detect the proportion of the intended genetic modification, the expression of HbF and persistence of edited cells.

6.4 Pharmacodynamics of CASGEVY

6.4.1 Persistence of Edited Cells (Proportion of Alleles with the Intended Genetic Modification) in Bone Marrow and Peripheral Blood

After CASGEVY infusion, the edited CD34⁺ cells engraft in the bone marrow and differentiate to erythroid lineage cells with reduced BCL11A expression. The persistence of edited cells was assessed by measuring the proportion of alleles with the intended genetic modification in CASGEVY drug product in peripheral blood and bone marrow over time using next-generation sequencing (NGS) method.

Table 4 summarizes the proportion of alleles with the intended genetic modification present in CD34⁺ cells of the bone marrow and peripheral blood over time in both primary efficacy set (PES) and full analysis set (FAS). In the FAS, the mean (SD) proportion of alleles with the intended genetic modification in CD34⁺ cells of bone marrow were 78.03% (11.63%) and 78.67% (12.64%) at Month 6 and Month 12 post-infusion, respectively. The mean proportion of alleles with the intended genetic modification in CD34⁺ cells of bone marrow remained stable (>75%) at Month 6 onward (Figure 3). Allelic editing in the peripheral blood was detectable within 1 month after CASGEVY infusion. The mean (SD) proportion of alleles with the intended genetic modification in peripheral blood was 50.15% (20.63%) at Month 1 and the mean remained stable (> 64%) from Month 2 onward (Figure 3). The small, non-zero value at baseline ($\leq 0.57\%$) was consistent with assay background signals. Allelic editing in the peripheral blood is lower than allelic editing in the CD34⁺ cells of the bone marrow because the peripheral blood includes lymphocytes that are not derived from the edited CD34⁺ hematopoietic stem cells (HSCs).

The PES showed similar profiles of allele editing over time in bone marrow and peripheral blood as that observed in the FAS (Table 4 & Figure 3).

Table 4. Summary of Proportion of Alleles (%) with Intended Genetic Modification in CD34⁺ Cells of the Bone Marrow and Peripheral Blood

a. PES		Bone Marrow (%)	Peripheral Blood (%)
Visit	Statistic	Total N = 35	Total N = 35
Baseline	n	--	35
	Mean (SD)	--	0.22 (0.12)
	Median	--	0.19
	Min, max	--	0.09, 0.57
Month 1	n	--	31
	Mean (SD)	--	44.08 (19.46)
	Median	--	49.01
	Min, max	--	0.91, 88.11
Month 3	n	--	34

	Mean (SD)	--	63.68 (10.88)
	Median	--	66.08
	Min, max	--	34.35, 84.38
Month 6	n	31	34
	Mean (SD)	75.57 (12.33)	63.90 (10.90)
	Median	78.26	66.81
	Min, max	41.77, 91.40	38.49, 78.47
Month 12	n	35	35
	Mean (SD)	77.21 (13.10)	65.67 (9.65)
	Median	83.83	68.65
	Min, max	34.98, 91.19	40.52, 77.85
Month 24	n	13	15
	Mean (SD)	75.43 (16.36)	64.93 (12.26)
	Median	81.34	66.46
	Min, max	45.02, 92.48	42.43, 82.81
Month 30	n	--	6
	Mean (SD)	--	65.95 (16.10)
	Median	--	72.56
	Min, max	--	40.29, 79.77
Month 36	n	--	2
	Mean (SD)	--	53.75 (17.61)
	Median	--	53.75
	Min, max	--	41.30, 66.20
Month 42	n	--	1
	Mean (SD)	--	66.63 (--)
	Median	--	66.63
	Min, max	--	66.63, 66.63

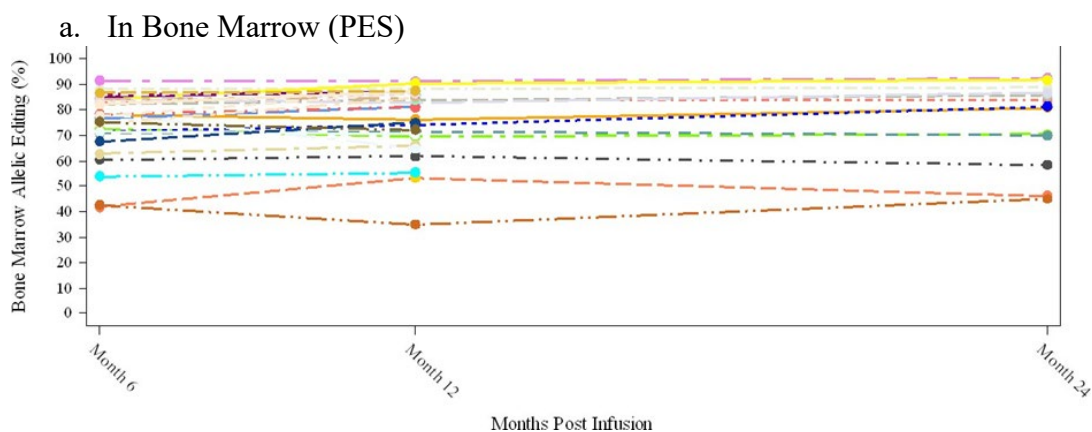
b. FAS

Visit	Statistic	Bone Marrow (%)	Peripheral Blood (%)
		Total N = 52	Total N = 52
Baseline	n	--	52
	Mean (SD)	--	0.21 (0.11)
	Median	--	0.18
	Min, max	--	0.09, 0.57
Month 1	n	--	46
	Mean (SD)	--	50.15 (20.63)
	Median	--	51.04
	Min, max	--	0.91, 88.11
Month 3	n	--	46
	Mean (SD)	--	66.21 (11.41)
	Median	--	66.67
	Min, max	--	34.35, 88.04
Month 6	n	41	44
	Mean (SD)	78.03 (11.63)	66.70 (11.29)
	Median	82.30	69.30
	Min, max	41.77, 91.40	38.49, 85.91
Month 12	n	41	43
	Mean (SD)	78.67 (12.64)	67.66 (10.16)

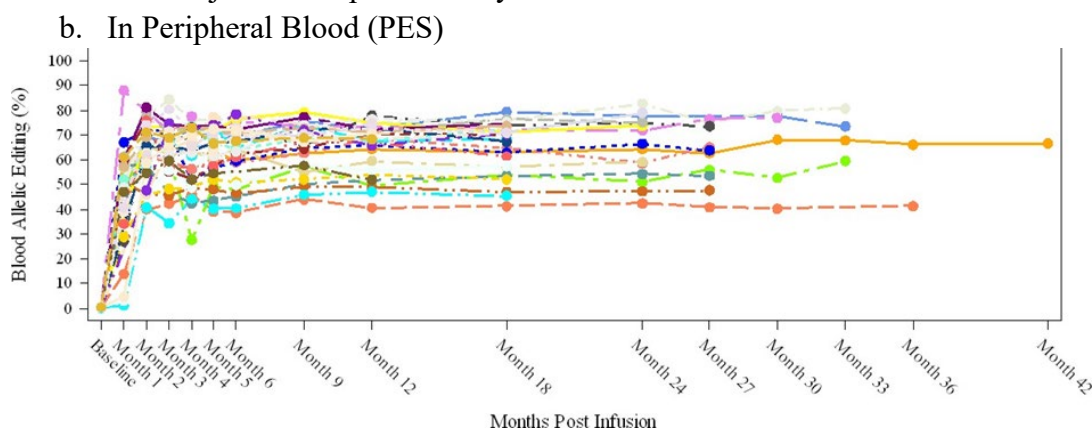
	Median	83.93	68.78
	Min, max	34.98, 91.19	40.52, 84.28
Month 24	n	13	15
	Mean (SD)	75.43 (16.36)	64.93 (12.26)
	Median	81.34	66.46
	Min, max	45.02, 92.48	42.43, 82.81
Month 30	n	--	6
	Mean (SD)	--	65.95 (16.10)
	Median	--	72.56
	Min, max	--	40.29, 79.77
Month 36	n	--	2
	Mean (SD)	--	53.75 (17.61)
	Median	--	53.75
	Min, max	--	41.30, 66.20
Month 42	n	--	1
	Mean (SD)	--	66.63 (--)
	Median	--	66.63
	Min, max	--	66.63, 66.63

Source: Applicant. Study CTX001-I31 CSR.

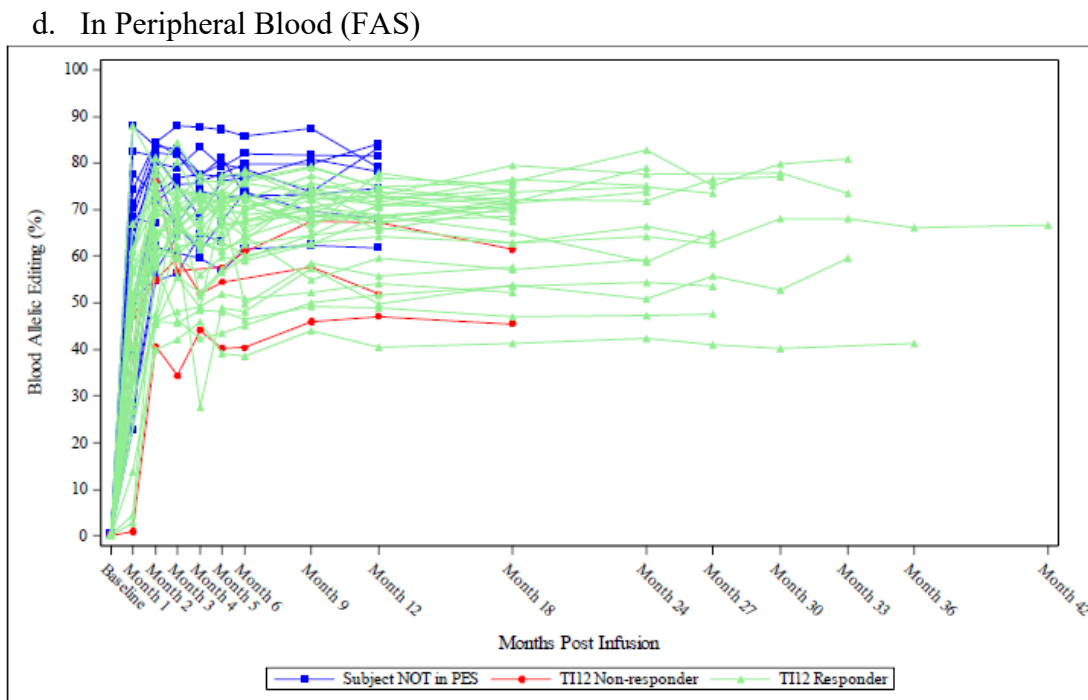
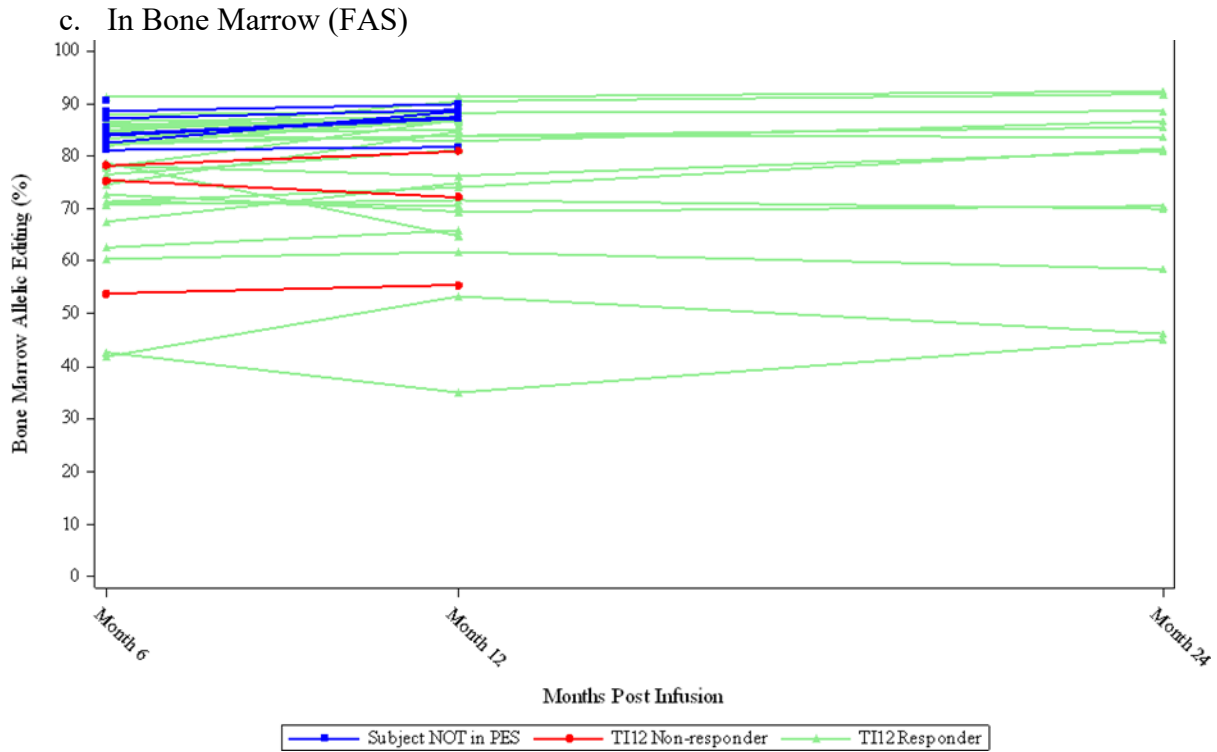
Figure 3. Individual Allelic Editing Over Time



*Individual subjects are represented by different colors.



*Individual subjects are represented by different colors.



Source: Applicant. Study CTX001-131 CSR.

6.4.2 Total Hemoglobin (Total Hb) and Fetal Hemoglobin (HbF) in Peripheral Blood

Levels of total Hb and HbF over time were measured. Total Hb levels were measured using the (b) (4) using the principles of ion-exchange (IEX) high performance liquid chromatography (HPLC). HbF levels were assessed using an HPLC assay. As shown in Table 5, in PES, increases in mean (SD) total Hb and HbF levels were observed as early as Month 3 after CASGEVY infusion and continued to increase to 11.9 (2.1) g/dL and 10.5 (3.0) g/dL respectively at Month 6. After Month 6, levels of total Hb and HbF were maintained thereafter, with HbF comprising $\geq 86\%$ of total Hb. The FAS showed similar profiles of total Hb and HbF as that observed in PES. In FAS, the mean (SD) total Hb and HbF levels were 12.2 (2.0) g/dL and 10.9 (2.8) g/dL respectively at Month 6. After Month 6, levels of total Hb and HbF were maintained thereafter, with HbF comprising $\geq 88\%$ of total Hb.

Table 5. Summary of Total Hb and HbF Concentrations and HbF(%) Over Time

a. PES

Visit	Statistic	Total Hb (g/dL) N = 35	HbF (g/dL) N = 35	HbF (%) ^a N = 35
Baseline	n	35	35	35
	Mean (SD)	10.4 (1.9)	0.5 (0.6)	5.5 (6.2)
	Median	10.1	0.3	3.4
	Min, max	6.9, 14.1	0.0, 2.2	0.0, 21.3
Month 3	n	34	33	33
	Mean (SD)	11.0 (2.3)	7.1 (3.1)	63.0 (21.4)
	Median	10.9	7.3	67.7
	Min, max	7.1, 17.6	0.3, 13.0	4.5, 93.8
Month 6	n	35	35	35
	Mean (SD)	11.9 (2.1)	10.5 (3.0)	86.8 (19.4)
	Median	12.3	11.4	95.2
	Min, max	6.5, 16.4	1.1, 14.5	16.4, 99.6
Month 9	n	35	34	34
	Mean (SD)	12.8 (2.2)	11.5 (2.9)	88.3 (14.4)
	Median	13.1	12.2	95.1
	Min, max	6.0, 17.5	2.5, 16.1	42.4, 100.0
Month 12	n	35	34	34
	Mean (SD)	12.7 (2.2)	11.4 (2.7)	89.0 (13.0)
	Median	12.9	12.3	96.2
	Min, max	6.2, 17.2	4.4, 15.3	49.5, 100.0
Month 18	n	30	27	27
	Mean (SD)	12.9 (2.1)	11.5 (2.4)	90.3 (10.8)
	Median	13.1	12.0	94.9
	Min, max	6.5, 17.7	4.3, 15.0	66.1, 100.0
Month 24	n	15	15	15
	Mean (SD)	13.2 (2.1)	11.0 (2.5)	89.2 (10.6)
	Median	13.5	12.1	93.5
	Min, max	10.1, 16.9	6.7, 15.4	68.8, 99.0

Month 30	n	6	6	6
	Mean (SD)	12.9 (0.9)	12.1 (0.9)	94.3 (5.0)
	Median	12.9	12.0	96.2
	Min, max	11.9, 14.3	11.0, 13.7	84.2, 97.1
Month 36	n	2	2	2
	Mean (SD)	13.3 (1.4)	12.6 (1.0)	95.2 (2.8)
	Median	13.3	12.6	95.2
	Min, max	12.3, 14.3	11.9, 13.3	93.2, 97.1
Month 42	n	1	1	1
	Mean (SD)	14.5 (--)	14.0 (--)	96.3 (--)
	Median	14.5	14.0	96.3
	Min, max	14.5, 14.5	14.0, 14.0	96.3, 96.3

b. FAS

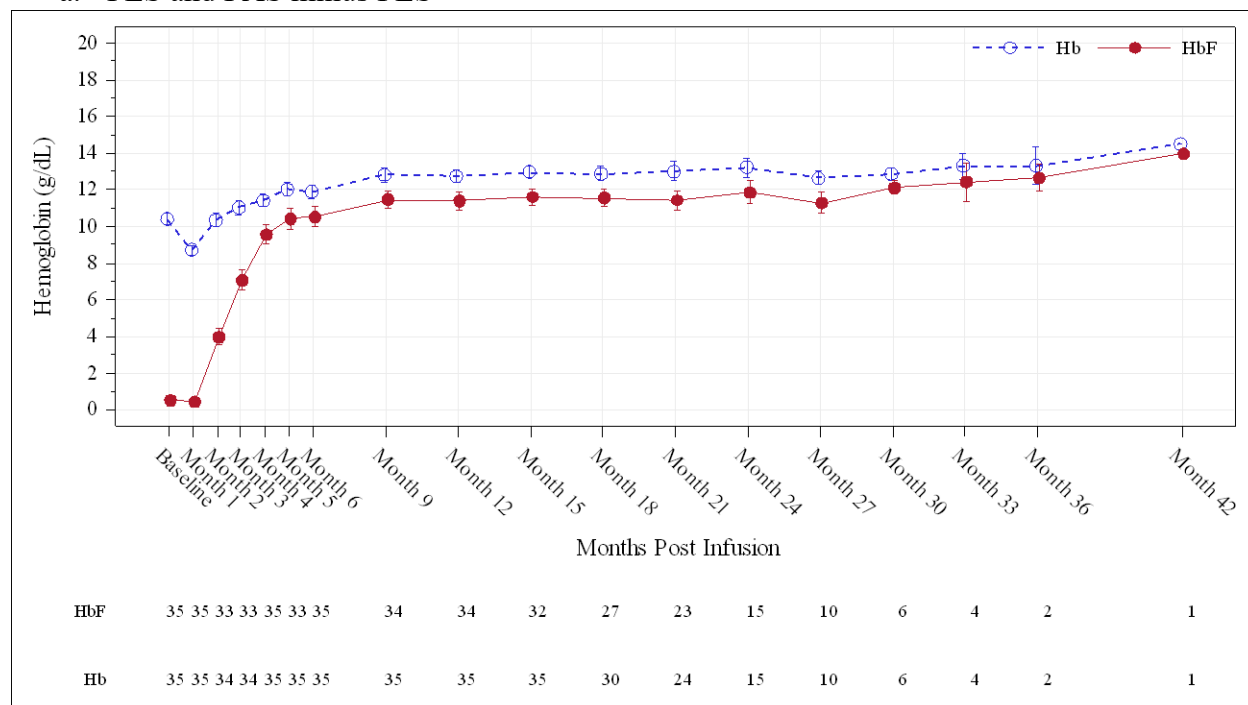
Visit	Statistic	Total Hb (g/dL) N = 52	HbF (g/dL) N = 52	HbF (%) ^a N = 52
Baseline	n	51	51	52
	Mean (SD)	10.4 (2.0)	0.6 (1.0)	6.6 (11.3)
	Median	10.1	0.3	3.4
	Min, max	6.9, 14.2	0.0, 5.8	0.0, 74.0
Month 3	n	47	46	46
	Mean (SD)	11.4 (2.2)	7.7 (2.9)	66.1 (19.6)
	Median	11.5	8.4	72.9
	Min, max	7.1, 17.6	0.3, 13.0	4.5, 93.8
Month 6	n	45	45	45
	Mean (SD)	12.2 (2.0)	10.9 (2.8)	88.3 (17.5)
	Median	12.5	11.6	95.2
	Min, max	6.5, 16.4	1.1, 14.5	16.4, 99.6
Month 9	n	44	43	43
	Mean (SD)	12.9 (2.1)	11.7 (2.6)	89.5 (13.3)
	Median	13.0	12.1	95.5
	Min, max	6.0, 17.5	2.5, 16.1	42.4, 100.0
Month 12	n	43	42	42
	Mean (SD)	12.8 (2.1)	11.5 (2.5)	89.7 (12.1)
	Median	12.9	12.3	96.2
	Min, max	6.2, 17.2	4.4, 15.3	49.5, 100.0
Month 18	n	30	27	27
	Mean (SD)	12.9 (2.1)	11.5 (2.4)	90.3 (10.8)
	Median	13.1	12.0	94.9
	Min, max	6.5, 17.7	4.3, 15.0	66.1, 100.0
Month 24	n	15	15	15
	Mean (SD)	13.3 (2.0)	12.0 (2.5)	89.1 (10.5)
	Median	13.5	12.4	93.5
	Min, max	10.1, 16.9	6.7, 15.4	66.8, 100.0
Month 30	n	6	6	6
	Mean (SD)	12.9 (0.9)	12.1 (0.9)	94.3 (5.0)
	Median	12.9	12.0	96.2
	Min, max	11.9, 14.3	11.0, 13.7	84.2, 97.1
Month 36	n	2	2	2
	Mean (SD)	13.3 (1.4)	12.6 (1.0)	95.2 (2.8)

	Median	13.3	12.6	95.2
	Min, max	12.3, 14.3	11.9, 13.3	93.2, 97.1
Month 42	n	1	1	1
	Mean (SD)	14.5 (--)	14.0 (--)	96.3 (--)
	Median	14.5	14.0	96.3
	Min, max	14.5, 14.5	14.0, 14.0	96.3, 96.3

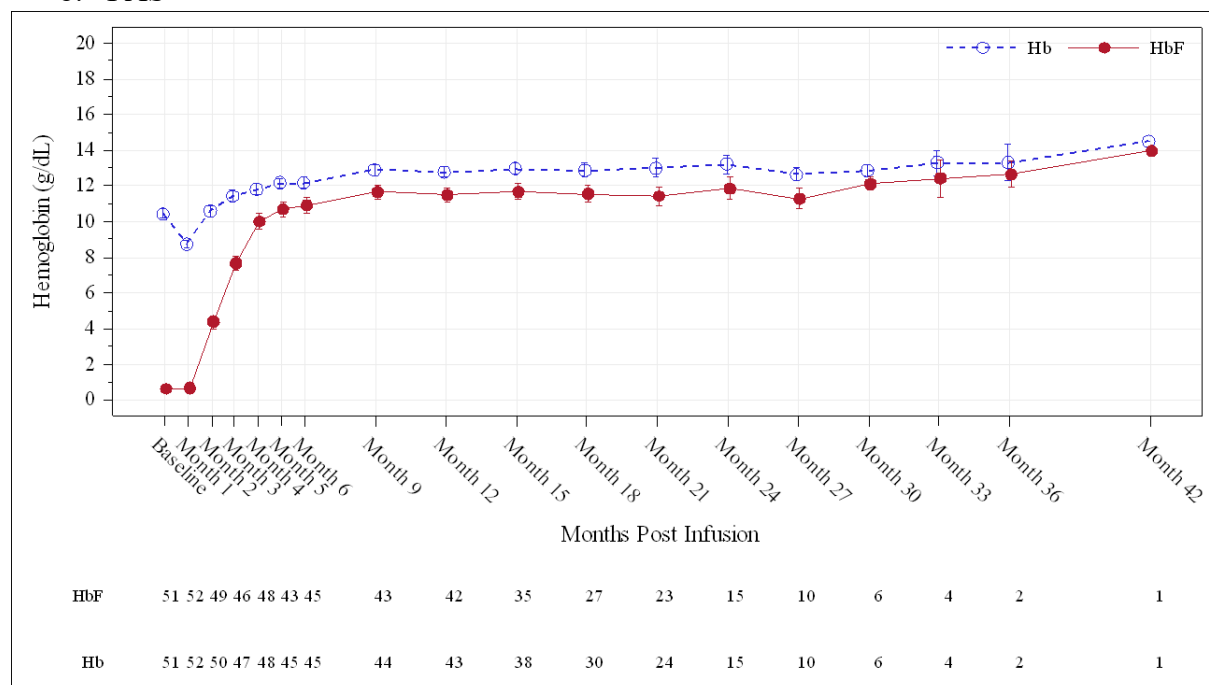
Source: Applicant. Study CTX001-131 CSR.

Figure 4. Summary of Mean Total Hb (g/dL) and HbF (g/dL) Over Time

a. PES and FAS minus PES



b. FAS



Source: Applicant. Study CTX001-131 CSR.

6.4.3 Proportion of F-Cells (%) in Peripheral Blood

The proportion of circulating erythrocytes expressing fetal hemoglobin (HbF), F-cells (%) was measured using flow cytometry assay. As shown in Table 6, in FAS, consistent with the increase in HbF levels, the mean (SD) F-cells (%) at Month 3 was 73.8% (19.7%) and continued to increase over time to 95.9% (15.2%) at Month 6, with levels remaining stable from thereafter, indicating sustained pan-cellular expression of HbF. Similar profiles were observed in PES (Table 6 & Figure 5).

Table 6. Summary of Proportion of F-Cells (%) Over Time

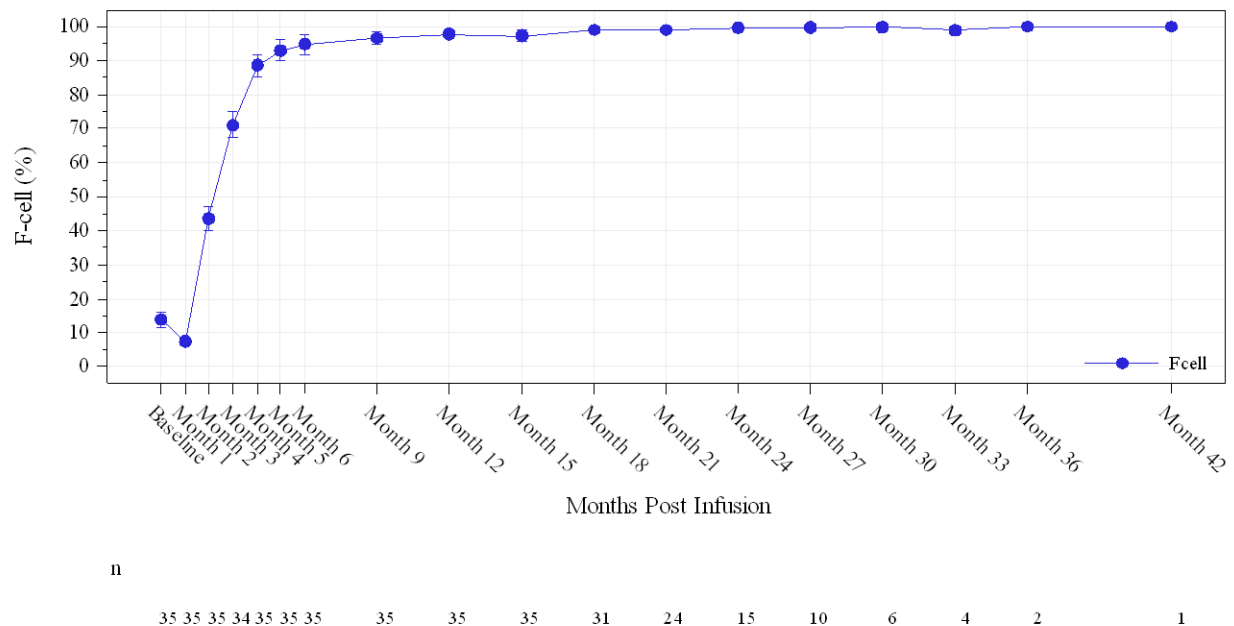
Visit	Statistic	PES	FAS
		Total (%) N = 35	Total (%) N = 52
Baseline	n	35	52
	Mean (SD)	13.97 (12.66)	14.07 (15.05)
	Median	8.80	8.60
	Min, max	3.0, 50.1	2.3, 83.9
Month 3	n	34	47
	Mean (SD)	71.03 (22.11)	73.82 (19.74)
	Median	80.15	81.00
	Min, max	6.0, 93.0	6.0, 93.3
Month 6	n	35	45
	Mean (SD)	94.79 (17.13)	95.91 (15.20)

	Median	99.80	99.80
	Min, max	25.7, 100.0	25.7, 100.0
Month 12	n	35	43
	Mean (SD)	97.77 (7.65)	98.15 (6.93)
	Median	99.80	99.90
	Min, max	57.0, 100.0	57.0, 100.0
Month 18	n	31	31
	Mean (SD)	99.03 (3.71)	99.03 (3.71)
	Median	99.90	99.90
	Min, max	79.3, 100.0	79.3, 100.0
Month 24	n	15	15
	Mean (SD)	99.59 (0.93)	99.59 (0.93)
	Median	99.90	99.90
	Min, max	96.3, 100.0	96.3, 100.0
Month 30	n	6	6
	Mean (SD)	99.95 (0.08)	99.95 (0.08)
	Median	100.00	100.00
	Min, max	99.8, 100.0	99.8, 100.0
Month 36	n	2	2
	Mean (SD)	100.00 (0.00)	100.00 (0.00)
	Median	100.00	100.00
	Min, max	100.00, 100.00	100.00, 100.00
Month 42	n	1	1
	Mean (SD)	100.00 (--)	100.00 (--)
	Median	100.00	100.00
	Min, max	100.00, 100.00	100.00, 100.00

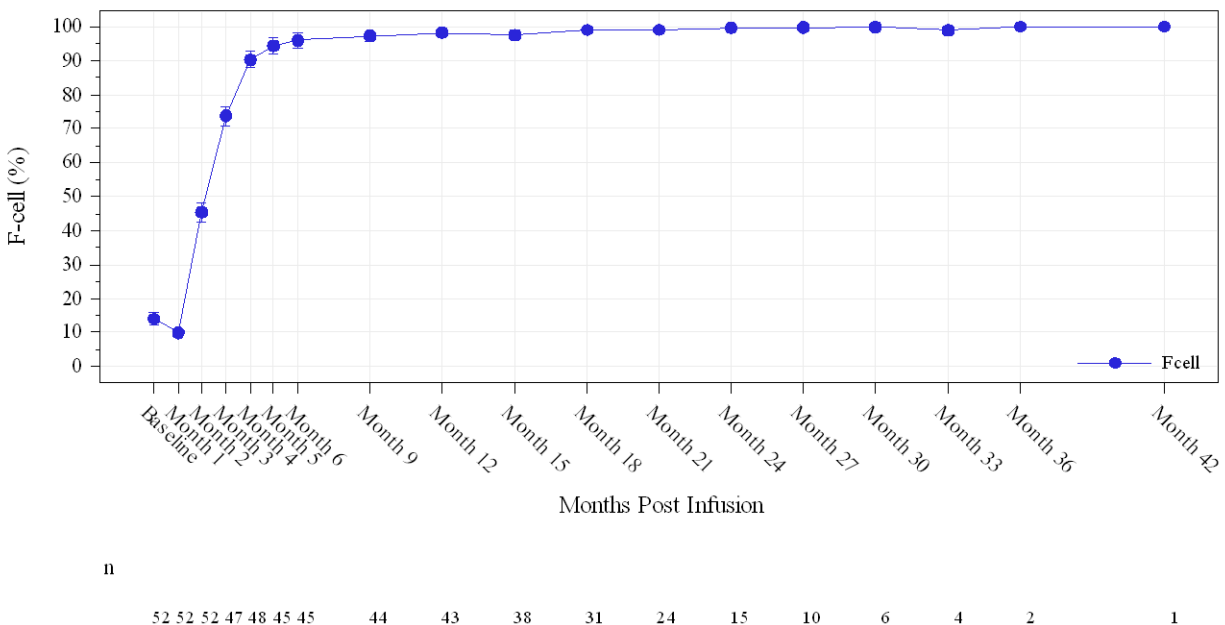
Source: Applicant. Study CTX001-131 CSR.

Figure 5. Summary of Proportion of F-Cells (%) Over Time

a. PES



b. FAS



Source: Applicant. Study CTX001-131 CSR.

6.4.4 Subgroup Analysis

Subgroup analyses evaluating the effects of age (adolescent versus adult), sex (male versus female), race, and genotype showed consistent results on total Hb, HbF and allelic editing in subjects with TDT. No significant impact of these intrinsic factors on the PD biomarkers was observed.

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

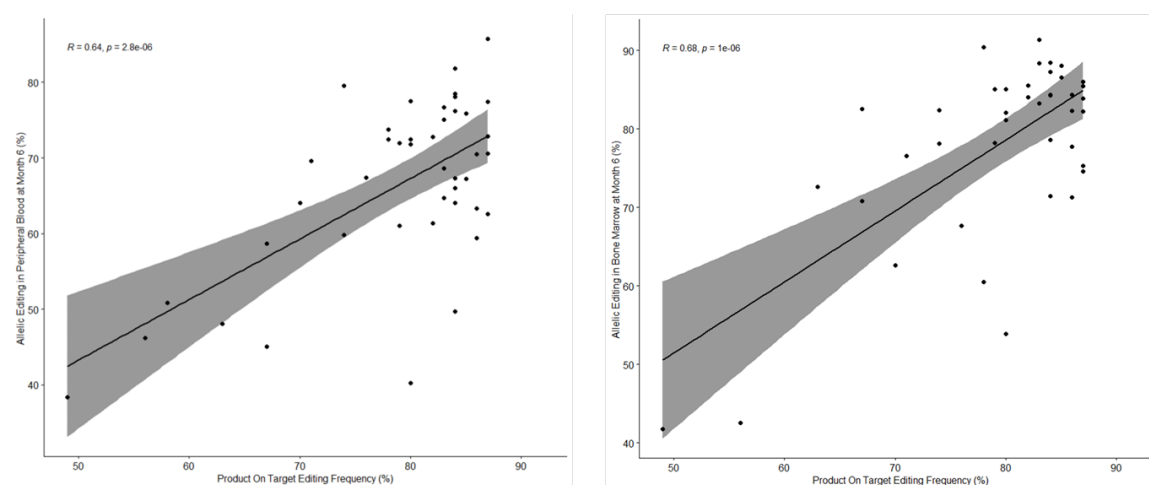
CASGEVY is an autologous gene therapy consisting of HSCs that have been genetically modified ex vivo and is intended for a single dose IV infusion. Considering the heterogeneity of CASGEVY product composition and one-time treatment dosing regimen, the product dosing characteristics were evaluated for the potential impacts on pharmacodynamic responses and clinical outcomes. Following CASGEVY product dosing characteristics were evaluated: CASGEVY body weight-based dose levels, administered total dose, on-target editing frequency (b) (4), the net increase in γ -globin expression (b) (4) and (b) (4).

6.5.1 Drug Product Dosing Characteristics and Pharmacodynamic Responses

Regression analysis indicated following potential associations between CASGEVY product dosing characteristics and PD responses ($p < 0.05$):

- Higher CASGEVY dose level (body weight-based) was potentially associated with higher proportion of allelic editing in bone marrow at Month 6 (Pearson corr. = 0.33, Spearman corr. = 0.42)
- Higher CASGEVY product on-target editing frequency was potentially associated with higher proportion of allelic editing in peripheral blood at Month 6 (Pearson correlation coefficient = 0.64, Spearman correlation coefficient = 0.40) and allelic editing in bone marrow at Month 6 (Pearson corr. = 0.68, Spearman corr. = 0.38).
- A potential negative trend was observed between the induction of the net increase of γ -globin expression ((b) (4)) and HbF% at Month 6 (Pearson corr. = -0.37, Spearman corr. = -0.6).
- A potential positive association was observed between total dose and allelic editing in bone marrow at Month 6 (Spearman corr. = 0.41)

Figure 6. Relationships between Product On-Target Editing Frequency and Allelic Editing in Bone Marrow (Left) and Peripheral Blood at Month 6



Source: Reviewer.

No additional potential correlations were observed. Due to the small sample size, the results should be interpreted with caution.

6.5.2 Drug Product Dosing Characteristics and Clinical Outcomes

No potential association was observed between CASGEVY product dosing characteristics and clinical outcome (time to achieve engraftment and transfusion independence).

6.6 Pharmacodynamic Responses and Clinical Outcomes

6.6.1 Relationships between Pharmacodynamic Parameters

Results of correlative analysis suggested following potential associations between pharmacodynamic biomarkers at Month 6 ($p < 0.05$ for both Pearson and Spearman correlation analysis) (Table 7):

Table 7. Summary of Relationships Between Pharmacodynamic Biomarkers at Month 6

Correlative Analysis	Sample Size	Pearson Correlation Coefficient	Spearman Correlation Coefficient
Allelic editing in bone marrow vs. Allelic editing in peripheral blood	40	0.72	0.63
Allelic editing in bone marrow vs. total Hb	41	0.38	0.41
Allelic editing in bone marrow vs. HbF	41	0.34	0.42
Allelic editing in peripheral blood vs. proportion of F-Cells (%)	44	0.34	0.33
Allelic editing in peripheral blood vs. total Hb	44	0.4	0.31
Allelic editing in peripheral blood vs. HbF	44	0.44	0.37
Total Hb vs. HbF	45	0.85	0.85
Total Hb vs. proportion of F-Cells (%)	45	0.52	0.32
HbF vs. HbF%	44	0.86	0.45
HbF vs. proportion of F-Cells (%)	45	0.76	0.47
HbF% vs. proportion of F-Cells (%)	44	0.88	0.43

Source: Reviewer.

The Applicant conducted both correlation analysis and population pharmacodynamic modeling to evaluate the relationships for total Hb, HbF, and the proportion of alleles with intended genetic modification in CD34+ cells of the bone marrow and peripheral blood between earlier and later time points for subjects in the [TDT]PES to predict durable efficacy of subjects who were not in the [TDT]PES.

Data at the earlier timepoint (Month 6) for Hb (g/dL), HbF (g/dL), and allelic editing (%) strongly correlated with the later timepoints (Months 12, 18, 21, 24), with correlation coefficient ≥ 0.8 . A strong correlation of the earlier timepoint (Month 6) with later timepoints was observed for all parameters including total Hb, HbF (g/dL), and allelic editing in bone marrow and peripheral blood for subjects in the [TDT]PES (Table 8). For each parameter, subjects with shorter follow-up (i.e., subjects who are not in the [TDT]PES) had similar results as subjects with longer follow-up (i.e.,

subjects in the [TDT]PES. These results support that subjects with shorter follow-up will also have similar durable efficacy as the subjects with longer follow-up.

Table 8. Summary of Correlation Between Different Visits for Selected Parameters for Subjects with TDT (Studies 111 & 131, [TDT]PES)

Parameter				
Correlation at Different Visits				
Statistic	Month 12	Month 18	Month 21	
	Month 24 Total Hb (g/dL)			
Pearson correlation between Month 6 Visit and a specific visit				
n	35	30	24	15
Correlation	0.91	0.88	0.81	0.83
HbF (g/dL)				
Pearson correlation between Month 6 Visit and a specific visit				
n	34	27	23	15
Correlation	0.95	0.90	0.80	0.82
Proportion of alleles with intended genetic modification in CD34 ⁺ cells of bone marrow (%)				
Pearson correlation between Month 6 Visit and a specific visit				
n	31	--	--	13
Correlation	0.93	--	--	0.98
Proportion of alleles with intended genetic modification in peripheral blood (%)				
Pearson correlation between Month 6 Visit and a specific visit				
n	34	31	--	15
Correlation	0.90	0.90	--	0.93

Source: Applicant. Study CTX001-131 CSR.

6.6.2 Pharmacodynamic (PD) responses and Clinical Outcomes

6.6.2.1 PD responses and Engraftment

Correlative analysis indicated following potential associations between pharmacodynamic biomarkers at Month 6 and engraftment ($p < 0.05$ for both Pearson and Spearman correlation analysis) (Table 9):

Table 9. Relationships between Pharmacodynamic Biomarkers and Engraftment

Correlative Analysis	Sample Size	Pearson Correlation Coefficient	Spearman Correlation Coefficient
Allelic editing in bone marrow vs. time to achieve neutrophil engraftment	41	-0.46	-0.48
Allelic editing in peripheral blood vs. time to achieve neutrophil engraftment	44	-0.39	-0.31
Total Hb vs. time to achieve neutrophil engraftment	45	-0.47	-0.36
HbF vs. time to achieve neutrophil engraftment	45	-0.48	-0.31

Source: Reviewer.

6.6.2.2 PD responses and Transfusion Independence

The primary efficacy endpoint of CASGEVY was based on achievement of transfusion independence 12 (TI12), which is defined as maintaining weighted average Hb ≥ 9 g/dL without RBC transfusions for at least 12 consecutive 12 months at any time after infusion of CASGEVY.

Table 10 is the contingency table assessing relationship between unsupported total Hb at Month 6 and TI12 status. All 30 subjects who had unsupported total Hb at Month 6 ≥ 9 g/dL achieved TI12. Among 5 subjects who had unsupported total Hb at Month 6 < 9 g/dL, 2 subjects achieved TI12.

Table 10. Contingency Table of Unsupported Total Hb at Month 6 versus Transfusion Independence (TI12)

	N	Achieved TI12	Did Not Achieve TI12
Do Not Have Unsupported Total Hb ≥ 9 g/dL at Month 6	5	2	3
Unsupported Total Hb ≥ 9 g/dL at Month 6	30	30	0

Total TI-evaluable subjects	35	32	3
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Source: Reviewer.

The HbF levels at Month 6 of the three subjects who did not achieve TI12 were lower than the HbF levels at Month 6 (> 6.9 g/dL) of other subjects who achieved TI12.

Reviewer's Comments:

Three subjects did not achieve TI12. One subject achieved platelet engraftment on Day 56 and had HbF of 6.77 g/dL at Month 6. Later improvement in total Hb and HbF levels was observed in this subject: HbF level of 7.72 g/dL and total Hb of 8.0 g/dL as of the last scheduled visit (Month 15) before the data cutoff date. Total Hb was 9.2 g/dL at the last unscheduled visit (Day 535).

Two of the subjects achieved platelet engraftment after more than 180 days post-infusion. The HbF levels at Month 6 were below 2.0 g/dL in the two subjects. HbF levels continued to increase with achievement of platelet engraftment. One subject had HbF level of 8.47g/dL at last scheduled visit (Month 21) and total Hb of 9.0 g/dL at the last unscheduled visit (Day 659). The other subject also showed improvement over time for HbF and total Hb levels: HbF level of 5.29 g/dL and total Hb of 6.9 g/dL as of the last scheduled visit (Month 21) before the data cutoff date. Total Hb was 7.4 g/dL at the last unscheduled visit (Day 638).

6.7 Clinical Pharmacology Conclusions

In subjects who received CASGEVY (full analysis set, FAS),

Persistence of Edited Cells: the mean proportion of alleles with the intended genetic modification in CD34+ cells of bone marrow remained stable ($>75\%$) from Month 6 onward. Allelic editing in the peripheral blood was detectable within 1 month and remained stable ($> 62\%$) from Month 2 onward.

Total Hemoglobin (total Hb) and Fetal Hemoglobin (HbF): increases in mean (SD) total Hb and HbF levels were observed as early as Month 3 after CASGEVY infusion and continued to increase to 12.2 (2.0) g/dL and 10.9 (2.8) g/dL respectively at Month 6. After Month 6, levels of total Hb and HbF were maintained thereafter, with HbF comprising $\geq 88\%$ of total Hb.

Proportion of Circulating Erythrocytes Expressing HbF (F-Cells): consistent with the increase in HbF levels, the mean (SD) proportion of circulating erythrocytes expressing HbF (F-cells) at Month 3 was 73.8% (19.7%) and continued to increase over time to 95.9% (15.2%) at Month 6, with levels remaining stable from thereafter, indicating sustained pan-cellular expression of HbF.

Durability of Pharmacodynamic Responses: the durability of pharmacodynamic responses was observed up to Month 42 post infusion of CASGEVY. Durability of PD responses was also supported by correlation analysis of PD biomarkers at different visits and population pharmacodynamic modeling.

Dose-Response Relationship: no dose-response relationship was identified for clinical efficacy (transfusion independence).

Total Hemoglobin Levels and Transfusion Independence (TI12): all 30 subjects who had unsupported total Hb at Month 6 of ≥ 9 g/dL achieved TI12. Among 5 subjects who had unsupported total Hb at Month 6 of < 9 g/dL, 2 subjects achieved TI12.

7 APPENDIX - INDIVIDUAL STUDY

7.1 Study #1 – Study CTX001-111

Interim Analysis 3 Data Cutoff Date: January 16, 2023

<p>Title: A Phase 1/2/3 Study of the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified CD34⁺ Human Hematopoietic Stem and Progenitor Cells (hHSPCs) in Subjects with Transfusion-dependent β-thalassemia</p>
<p>Objectives:</p> <p>Primary Objective:</p> <p>To evaluate the safety and efficacy of a single dose of autologous CRISPR/Cas9 modified CD34⁺ hHSPCs (exagamglogene autotemcel [exa-cel], formerly CTX001) in subjects with transfusion-dependent β-thalassemia (TDT)</p> <p>Secondary Objectives:</p> <ul style="list-style-type: none"> • To quantify percentage of edited alleles in peripheral blood leukocytes and CD34⁺ cells of the bone marrow • To assess the production of fetal hemoglobin (HbF) after exa-cel infusion • To assess the effects of infusion of exa-cel on disease-specific events and clinical status <p>Exploratory Objectives:</p> <ul style="list-style-type: none"> • To assess the ability of biomarkers to characterize exa-cel effect and predict treatment outcomes
<p>Study Design</p> <p>This is a single-arm, open-label, multi-site, single dose, Phase 1/2/3 study in subjects 12 to 35 years of age (inclusive) who have TDT. Transfusion dependence was defined as a history of at least 100 mL/kg/year or 10 units/year of packed red blood cell (RBC) transfusions in the 2 years before the signing the informed consent form (ICF). The study evaluated the safety and efficacy of a single dose of exa-cel.</p> <p>For each subject, the study was conducted in 4 stages: Stage 1 included Screening and the pre-mobilization period, Stage 2 included mobilization, autologous CD34⁺ stem cell collection, and exa-cel manufacture and disposition, Stage 3 included myeloablative conditioning and exa-cel infusion, and Stage 4 included post-infusion in-hospital follow-up (until neutrophil engraftment) and post-discharge follow-up (approximately 2 years). All subjects who received exa-cel were offered enrollment into the long-term follow-up study (Study 131) after completion or withdrawal/discontinuation from Study 111.</p>

Number of Subjects:

Approximately 45 subjects were planned to be dosed with exa-cel. A total of 59 subjects were enrolled at the time of the interim analysis (IA)³; 52 subjects were infused with exa-cel.

Analysis Set	Total
Enrolled Set^a	59
Safety Analysis Set^b	59
FAS^c	52
PES^d	35
EES^e	43

Diagnosis and Main Criteria for Inclusion:

Subjects 12 to 35 years of age (inclusive) with TDT as defined by:

- Documented homozygous β -thalassemia or compound heterozygous β -thalassemia including β -thalassemia/hemoglobin E (HbE).
- A history of at least 100 mL/kg/year or 10 units/year of packed RBC transfusions 2 years before signing the ICF or the last rescreening (if applicable).

Study Treatments**Investigational medicinal product(s): CASGEVY (exagamglogene autotemcel, exa-cel)**

The median dose of exa-cel was 7.5 (range: 3.0 to 19.7) $\times 10^6$ CD34⁺ cells/kg by intravenous (IV) infusion.

Formulation: cell suspension

Route(s) of administration: intravenous (IV)

Dose regimen: single dose

Clinical Pharmacology Sampling Time PointsBusulfan Pharmacokinetics:

For once daily dosing: end of the 3-hour infusion (3-hours) (± 5 minutes), 3 hours 15 minutes (± 5 minutes), 3 hours 30 minutes (± 5 minutes), 4 hours (± 15 minutes), 5 hours (± 15 minutes), 6 hours (± 15 minutes), and 8 hours (± 15 minutes).

For dosing q6h: end of the first 2-hour infusion (2-hours) (± 5 minutes), 2 hours 15 minutes (± 5 minutes), 2 hours 30 minutes (± 5 minutes), 3 hours (± 15 minutes), 4 hours (± 15 minutes), 5 hours (± 15 minutes), and 6 hours (± 15 minutes).

Pharmacodynamic Sampling Times

For Blood Samples: Baseline (prior to mobilization start), Month 1 (± 4 d), Month 2 (± 7 d), Month 3 (± 7 d), Month 4 (± 7 d), Month 5 (± 7 d), Month 6 (± 14 d), Month 9 (± 14 d), Month 12 (± 14 d), Month 15 (± 14 d), Month 18 (± 30 d), Month 21 (± 30 d), and Month 24 (± 30 d).

For Bone Marrow Samples: Month 6 (± 14 d), Month 12 (± 14 d), and Month 24 (± 30 d)

Pharmacokinetic Results for Busulfan

- Myeloablation as performed in this study was adequate and sufficient because all 52 subjects who received exa-cel after busulfan conditioning achieved profound neutropenia and engraftment of edited cells, with stable allelic editing over time.
- For the 53 subjects who received busulfan, the mean (SD) administered busulfan dose was 3.54 (0.481) mg/kg/day for the every 6 hours (q6h) regimen and 3.88 (0.742) mg/kg/day for the once daily (qd) regimen.
- Of the 53 subjects who received busulfan, the majority of subjects for the q6h regimen (74%) and for the qd regimen (53%) were within the protocol-specified busulfan cAUC target range.
- For the 52 subjects who received exa-cel, no clinically relevant impact on exa-cel efficacy was noted for subjects with busulfan cAUC outside the protocol-specified cAUC target range. The safety of myeloablative busulfan conditioning in the subjects who received busulfan (N = 53) was consistent with the known safety

profile of busulfan (Busulfan USPI).

- No clinically relevant effects of age at screening, sex, race, genotype, or weight on observed busulfan cAUC were observed.

Pharmacokinetic/Pharmacodynamic Results for Busulfan

There were no clinically relevant effects of busulfan cAUC or busulfan dosing regimen (q6h or qd) on time to neutrophil or platelet engraftment.

Pharmacodynamic Results:

Total Hb and HbF

- For subjects in the PES, after exa-cel infusion, increases in mean total Hb and HbF levels occurred early (Month 3) and were maintained over time (from Month 6 onward).
 - Mean total Hb levels were 11.0 g/dL at Month 3, increased to 11.9 g/dL at Month 6, and were maintained ≥ 11 g/dL over the duration of follow-up.
 - Mean HbF levels were 7.1 g/dL at Month 3, increased to 10.5 g/dL at Month 6, and were thereafter maintained ≥ 10 g/dL over the duration of follow-up.
 - The mean proportion of F-cells (an exploratory endpoint) was 71.03% at Month 3, increased to 94.79% at Month 6, and was maintained $\geq 94\%$ for the duration of follow-up.
- For subjects in the EES and the FAS, including the subset of subjects who were not yet eligible to be part of the PES, increases in mean total Hb and HbF levels occurred early (at Month 3) after exa-cel infusion, and were consistent with the PES.

Proportion of Alleles With Intended Genetic Modification

- For subjects in the PES: Allelic editing data in CD34⁺ cells of the bone marrow and peripheral blood were indicative of the durable engraftment of edited long-term hematopoietic stem cells (HSCs) and reflect the permanent nature of the intended edit. Percent allele editing in individual subjects was stable over time during the follow-up period through Month 24.
 - CD34⁺ cells of the bone marrow: At Month 6 (first timepoint of evaluation), the mean proportion of alleles with the intended genetic modification was 75.57% in CD34⁺ cells of the bone marrow, which was consistent with what was observed in the proportion of alleles with intended genetic modification in the exa-cel drug product (mean: 86.41%). The mean proportion of alleles with the intended genetic modification in bone marrow remained stable at Month 12 ($\geq 75\%$) onward.
 - Peripheral blood: Allelic editing in the peripheral blood was detectable within 1 month after exa-cel infusion. The mean proportion of alleles with the intended genetic modification in peripheral blood was 44.08% at Month 1 and the mean remained $\geq 62\%$ from Month 2 onward.
- For subjects in the EES and the FAS, including the subset of subjects who were not yet eligible to be part of the PES, the proportion of alleles with the intended genetic modification in CD34⁺ cells of the bone marrow and peripheral blood were consistent with the PES.

Subgroup Analyses

The proportion of subjects who achieved TI12 and TI6 was similar for subjects ≥ 12 and < 18 years of age and ≥ 18 and ≤ 35 years of age and was similar across genotype (β^0/β^0 -like and non- β^0/β^0 -like), race (Asian, White, and other races), and sex. Although some subgroups were small, the results of the subgroup analyses (age, genotype, race, and sex) were consistent with the results from the main analyses of the primary, key secondary, and secondary efficacy endpoints. This is consistent with a common pathophysiology across these subgroups and the common mechanism of action of exa-cel in HbF reactivation.

Conclusions (Clinical pharmacology-related)

- For subjects in the PES, clinically meaningful increases in mean total Hb, HbF, and F-cell levels were demonstrated early (at Month 3) after exa-cel infusion and were maintained over time from approximately Month 6 onward, demonstrating the achievement of hematologic stability, consistent with absence of and/or reduction in transfusions. For subjects in the FAS, including the subset of subjects who were not yet eligible to be part of the PES, increases in mean total Hb, HbF, and F-cell levels occurred early (at Month 3) after exa-cel infusion, and were consistent with the PES.
- For all subjects in the PES, a high, stable proportion of alleles with the intended genetic modification was observed in both the CD34⁺ cells of the bone marrow and peripheral blood, indicating durable engraftment of edited long-term HSCs and reflecting the permanent nature of the intended edit. For subjects in the FAS, including the subset of subjects who were not yet eligible to be part of the PES, the proportion of alleles with the intended genetic modification in CD34⁺ cells of the bone marrow and peripheral blood were consistent with the PES.

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

7.2 Study #2 – Study CTX001-131

Interim Analysis Data Cutoff Date: January 16, 2023 (for subjects from Study 111)

<p>Title: A Long-term Follow-up Study of Subjects with β-thalassemia or Sickle Cell Disease Treated with Autologous CRISPR-Cas9 Modified Hematopoietic Stem Cells (CTX001)</p>
<p>Objectives:</p> <p>Primary Objectives: Evaluate long-term safety up to 15 years after exagamlogene autotemcel (exa-cel; formerly CTX001) infusion, in subjects who received exa-cel.</p> <p>Secondary Objectives: To evaluate efficacy of exa-cel up to 15 years after exa-cel infusion, in subjects who received exa-cel for treatment of transfusion-dependent β-thalassemia (TDT) or severe sickle cell disease (SCD).</p>
<p>Methodology: This is an ongoing, multi-site, open-label, rollover study designed to evaluate the long-term safety and efficacy of exa-cel in subjects who received exa-cel in a parent study for a total follow-up of 15 years after exa-cel infusion. Parent studies initiated to date include Studies 111 and 141 in subjects with TDT and Studies 121 and 151 in subjects with severe SCD. Studies 111 and 121 are the first-in-human Phase 1/2/3 studies with exa-cel in subjects 12 to 35 years of age. Studies 141 and 151 are ongoing studies in subjects 2 to 11 years of age. In each of the 4 parent studies, subjects are followed for up to approximately 2 years (to Month 24) after exa-cel infusion. All subjects who received exa-cel infusion who completed or discontinued from a parent study were asked to participate in Study 131. No subjects from Study 141 or Study 151 are included in the analyses as of the current data cutoff dates. Therefore, methods and endpoints unique to these studies are not described in this interim report.</p>
<p>Number of Subjects: A total of up to 114 subjects treated for TDT or severe SCD are expected to be enrolled in this ongoing study. As of the January (TDT subjects)/February (SCD subjects) 2023 data cutoff dates, the TDT and SCD analysis sets are based on subjects from parent Study 111 (TDT) and parent Study 121 (SCD). Because these studies remain ongoing, only a subset of subjects have completed the parent studies and enrolled in Study 131. The analysis periods are presented as time after exa-cel infusion, including time in the respective parent study (Study 111 or 121) and the long-term follow-up (Study 131). Therefore, the analysis sets include all subjects from the parent study, including those who have not yet enrolled in Study 131. As of the current data cutoff dates, 14 subjects from Study 111 and 8 subjects from Study 121 have enrolled in Study 131.</p>
<p style="text-align: center;">Subjects From Parent Study</p>

Analysis Set	Study 111	Study 121
[TDT]Enrolled Set ^a	59	0
[TDT]SS ^b	59	0
[TDT]FAS ^c	52	0
[TDT]PES ^d	35	0
[SCD]Enrolled Set ^a	0	63
[SCD]SS ^b	0	58
[SCD]FAS ^c	0	42
[SCD]PES ^d	0	20
Diagnosis and Main Criteria for Inclusion: All subjects who completed or discontinued from a parent study after exa-cel infusion were asked to participate in this long-term follow-up study (Study 131). The current analyses include subjects from Studies 111 and 121. <ul style="list-style-type: none"> Subjects from Study 111 had homozygous or compound heterozygous β-thalassemia and a history of at least 100 mL/kg/year or 10 units/year of packed red blood cell (RBC) transfusions in the 2 years prior to screening. Subjects from Study 121 had a β^S/β^S, β^S/β^0, or β^S/β^+ genotype and severe SCD as defined by at least 2 vaso-occlusive crises (VOCs) per year during the 2-year period before screening. 		
Study Treatments N/A		
SUMMARY OF RESULTS As of the January (TDT subjects)/February (SCD subjects) 2023 data cutoff dates, 14 subjects with TDT completed Study 111 and 8 subjects with SCD completed Study 121, all of whom have enrolled in Study 131. No subjects discontinued from Study 131.		
Demographics and Other Baseline Characteristics <u>Subjects with TDT</u> Of the 14 subjects who rolled over to Study 131 from Study 111, 9 were female and 5 were male. Six subjects had β^0/β^0 -like genotypes (2 IVS-I-110/IVS-I-110, 2 IVS-I-110/ β^0 , and 2 β^0/β^0), and 8 subjects had non- β^0/β^0 -like genotypes (3 β^+/ β^+ , 3 β^+/β^0 , and 2 β^E/β^0). Subject ages ranged from 18 to 32 years. The baseline annualized units of TDT-related RBC transfusions ranged from 20.5 to 61.0 units per year, with a baseline annualized volume of 125.65 to 307.27 mL/kg per year, for the prior 2 years before screening in the parent study.		
Pharmacodynamic Results: <u>Subjects with TDT</u> <ul style="list-style-type: none"> For subjects who rolled over to Study 131, the increases in mean total Hb and HbF levels observed in Study 111 from Month 6 were stable and continued to be maintained after Month 24, with mean total Hb levels ≥ 11 g/dL and mean HbF levels ≥ 10 g/dL. Consistent with increases in HbF, the mean proportion of F-cells (an exploratory endpoint evaluating pancellularity of HbF) was maintained at $\geq 94\%$ from Month 6 through the duration of follow-up. For subjects who rolled over to Study 131, the mean proportion of alleles with the intended genetic modification in peripheral blood observed in Study 111 was stable and generally continued to be maintained with mean $\geq 62\%$ after Month 24 through the duration of follow-up. The mean values reflected stable levels across Studies 111 and 131 on an individual basis. In the [TDT]PES, measures of total Hb, HbF (g/dL), and allelic editing in peripheral blood and bone marrow at Month 6 correlated with measures at later timepoints through Month 24, with correlation coefficient ≥ 0.8. In addition, Month 6 results for these parameters were similar for subjects who had shorter (i.e., not in [TDT]PES) and longer (i.e., in [TDT]PES) follow-up. Together, these results support that subjects with 		

shorter follow-up will have similar durable efficacy as the subjects with longer follow-up.

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

7.3 Study #3 – Population Pharmacodynamic Analysis (Study # T067)

The Applicant developed population pharmacodynamic (PD) model to 1) characterize the longitudinal changes in HbF and total Hb (tHb) following CASGEVY administration in TDT subjects; and 2) predict HbF and tHb levels for all subjects analyzed as expected at Month 24 and through the duration of the follow-up.

(b) (4)

were used in PopPD analysis.

7.3.1 Data Source

Population PD models were built using data from Studies CTX001-111 and CTX001-131. The HbF analysis dataset included a total of 485 observations for 48 adolescent and adult TDT subjects, 12 – 35 years of age. No limit of quantification caused loss of HbF data. The duration of HbF data from subjects ranged from 4 to 42 months, providing between 4 and 17 HbF samples per subject to the analysis.

7.3.2 Methods

7.3.2.1 Structural Model

Base model development to describe the pharmacology of CASGEVY consisted of population longitudinal non-linear mixed-effects (NLME) repeated-measures models for HbF levels in TDT subjects from studies 111 and 131. Based on graphical inspections, a sigmoidal Emax model was used as the fixed effects structure, as in below equation:

$$HbF = HbF_{T0} + \frac{(HbF_{max} - HbF_{T0}) \times Time^{\gamma}}{Time^{\gamma} + T_{50}^{\gamma}}$$

where:

- dependent variable HbF is fetal hemoglobin (g/dL);
- $Time$ is the duration (in days) since the first observation after CASGEVY dosing;
- HbF_{T0} is HbF (g/dL) at the first observation after CASGEVY dosing
- HbF_{max} is the maximum HbF (g/dL);
- T_{50} is the duration (in days) needed to reach 50% of HbF_{max}
- γ is the Hill coefficient reflecting the sigmoidicity around T_{50}

Fitting of the model required considerations of which HbF data was appropriate to be used as time-zero values. A longitudinal graphical analysis of the data revealed that the median time between baseline HbF observations and CASGEVY administration was 174 (range: 104 – 750) days and that HbF levels were highly variable. These observations, together with pre-treatment regimens required prior to CASGEVY dosing (cellular mobilizations and myeloablation), resulted in the baseline HbF values as collected not being considered reliable representations of HbF levels at, or immediately following, CASGEVY administration. Therefore, the earliest post-dose timepoint (26 days for Subject (b) (4) ID (b) (6)) was selected to represent time-zero for modeling. While HbF levels at this timepoint are affected by the previous administration of CASGEVY, the Applicant stated it's more closely represent the baseline condition relative to dose administration. Therefore, Day 26 (the earliest post-CASGEVY HbF observation across all subjects) was used to align first observations per subject to time-zero and subsequently estimate HbF_{T0} .

7.3.2.2 Random Effects

Inter-individual (IIV) variability in each model parameter, P , was included with transformations informed by the distributions of the observed data and iterative evaluations during modeling. Several between-subject variance models were considered for the parameters of the model. Those tested included exponential and additive error models.

For model parameters with physiologically determined value limits (e.g. maximum effect), such as HbF_{max} and HbF_{T0} , estimation of the parameter and IIV within the logit domain was evaluated, using the form:

$$P_i = (1 + e^{-1 \times (P_0 + \eta_{P_i})})^{-1} \times 18$$

where P_i is the estimated parameter for subject i , P_0 is the typical population value of HbF_{max} and HbF_{T0} in the logit domain. The η_{P_i} are individual-specific inter-individual random effects for individual i and parameter P , and are assumed to be normally distributed according to $\eta \sim N(0, \Omega)$. Maximum normal tHb of 18 g/dL was used.

The resulting IIVs were examined graphically to assess adherence to the normality assumptions (histograms of random effect) and independence (scatterplot of each random effect against all of the others) assumptions. Once the IIV parameterizations were selected, residual variability model structures were evaluated. An additive error model best described the data and was selected.

7.3.2.3 Endogenous non-HbF Hb estimates

To provide predictions of tHb, the endogenous production of non-HbF Hb (hereafter eHb) was determined from the observed data and added to the model HbF predictions. Hb and HbF data 6 months post CASGEVY administration and 60 days after a subject's last transfusion were used to calculate the median eHb. The median eHb for each subject was determined as the difference between time-matched tHb and HbF samples. For subjects where eHb was incalculable due to data paucity or continuing transfusions, the median of the log transformed distribution of eHb across all qualifying subjects was used.

7.3.2.4 Model Diagnosis

Goodness of fit and visual predictive check (VPC) were used for model diagnostics.

7.3.2.5 Mode-based Simulations

The base model was used to predict in-cohort individual and population typical HbF following exa-cel administration, as well as tHb, at timepoints of interest. The expected in-cohort HbF was predicted for all subjects included in the analysis and out to month 48. tHb was predicted using the individual model predicted HbF plus the individual eHb. In subjects for whom eHb was incalculable, the median of the log transformed cohort values was imputed.

7.3.3 Results

7.3.3.1 Population PD Model

The HbF base model (sigmoidal Emax model) parameters were summarized in Table 11. The diagnostic plots and visual predictive checks (VPC) are presented in Figure 7 and Figure 8. The VPC of the model indicated that the base model captured the majority of the observed data well. Two subjects (subjects (b) (6) and (b) (6)) had different trajectories from the rest, their data was below the 2.5th percentile of the VPC. The Applicant reran the model excluding the two subjects, and the subsequent VPC demonstrated that the 2.5th percentile of the remaining observed data was well described by the VPC-generated 2.5th percentile (Figure 9).

For endogenous non-HbF Hb estimates (eHb), of the 48 subjects included in modeling, 43 had HbF levels that stabilized and were 60 days beyond their last transfusion as of month 6. Five did not have HbF levels through month 6 or had not accrued data 60 days after their last transfusion. In those that met the above criteria, the eHb distribution was approximately log normal with median eHb of 0.594 g/dL (range: 0.0375 - 4.38 g/dL) (Figure 10). Total Hb (tHb) was able to be estimated by the sum of eHb and model predicted HbF to forecast tHb at later time points than currently observed.

Reviewer's Comments:

The Applicant's sigmoidal Emax describing the HbF levels after infusion of CASGEVY in TDT subjects from Studies CTX001-111 and CTX001-131 is reasonable. During the model development and diagnosis, it was noticed that two subjects (subjects (b) (6) and (b) (6)) had different trajectories from the rest. When rerunning the model without the two subjects, the 2.5th percentile of the remaining observed data was well described by the VPC-generated 2.5th percentile. It was observed that the two subjects also had longer time to achieve platelet engraftment (> 180 days post CASGEVY infusion) and this may be considered. The impact of delayed platelet engraftment (e.g. > 180 days) on modeling of the HbF levels may be explored with more observations in future if available.

Table 11. HbF NLME Base Model Parameters

		Estimate	RSE%	Correlation	[95% CI]
Structural model parameters					
θ_1	Typical value of scaled logit HbF_{max}	0.758 [†]	11	-	[0.594, 0.921]
θ_2	Typical value of scaled logit HbF_{T0}	-3.73 [†]	6.8	-	[-4.23, -3.23]
θ_3	Typical value of T_{50} (days)	49.7	6.82	-	[43, 56.3]
θ_4	Typical value of γ	2.47	4.31	-	[2.26, 2.67]
Inter-individual variances					
$\Omega_{1,1}$	Variance of scaled logit HbF_{max}	0.302 [†]	24.7	-	[0.156, 0.449]
$\Omega_{2,2}$	Variance of scaled logit HbF_{T0}	1.32 [†]	39.1	-	[0.309, 2.33]
$\Omega_{3,3}$	Variance of T_{50}	0.208 [‡]	40.8	-	[0.0418, 0.374]
$\Omega_{4,4}$	Variance of γ	0.332	41.9	-	[0.0593, 0.605]
Inter-individual covariances					
$\Omega_{1,2}$	Covariance of HbF_{max} , HbF_{T0}	0.0138	-	0.0219	[-0.253, 0.28]
$\Omega_{1,3}$	Covariance of HbF_{max} , T_{50}	-0.0629	-	-0.251	[-0.184, 0.0583]
$\Omega_{1,4}$	Covariance of HbF_{max} , γ	-0.0188	-	-0.0593	[-0.16, 0.122]
$\Omega_{2,3}$	Covariance of HbF_{T0} , T_{50}	-0.465	-	-0.888	[-0.835, -0.0957]
$\Omega_{2,4}$	Covariance of HbF_{T0} , γ	0.0704	-	0.106	[-0.264, 0.405]
$\Omega_{3,4}$	Covariance of T_{50} , γ	0.00198	-	0.00754	[-0.105, 0.109]
Residual error variance					
Σ	Variance of additive residual error	0.435	14	-	[0.315, 0.555]

RSE: Relative Standard Error CI:
confidence interval

HbF_{max} : maximum HbF (g/dL)

HbF_{T0} : HbF (g/dL) at first observation post exacer dosing

T_{50} : duration (in days) needed to reach 50% of HbF_{max}

γ : Hill coefficient reflecting the sigmoidicity around T_{50}

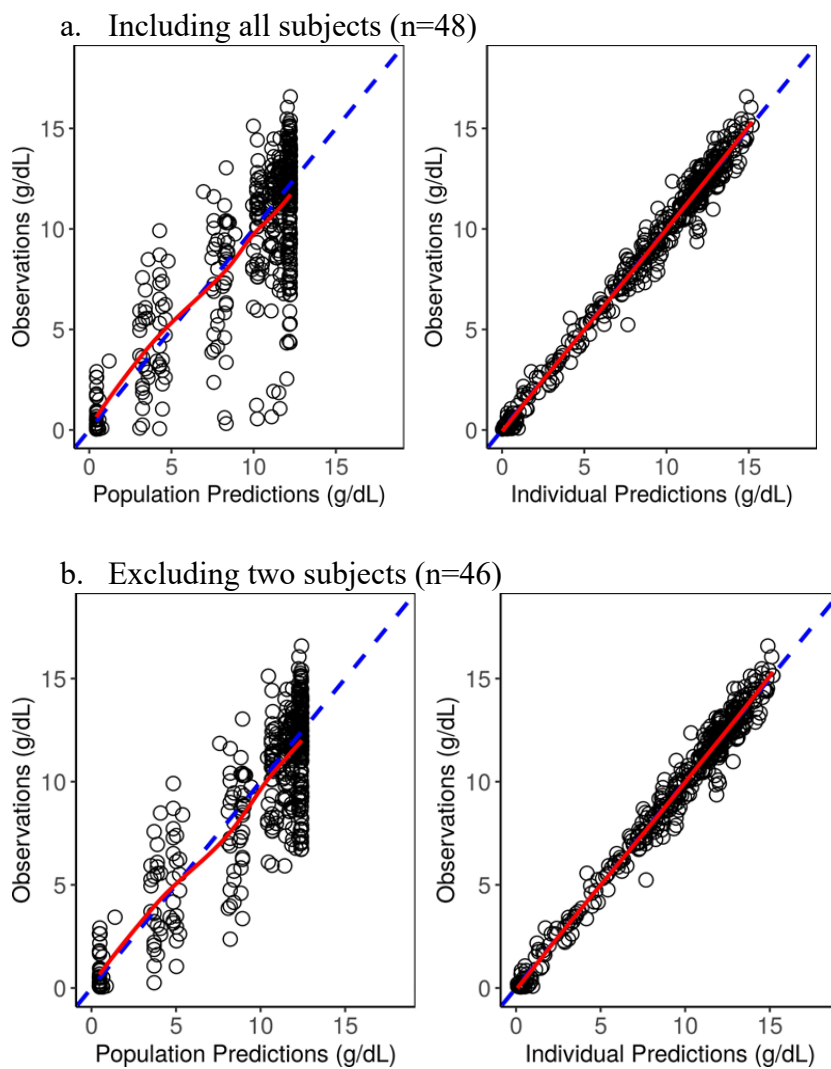
[†]Estimates and summary statistics for θ_1 , θ_2 , and their respective variances, $\Omega_{1,1}$, $\Omega_{2,2}$, are reported in the scaled logit domain (see (Equation 6.4)).

[‡]Estimates and summary statistics for $\Omega_{3,3}$ are reported in the logarithmic domain (see (Equation 6.2)).

Correlation between variances (COR) is calculated as $COR(X, Y) = \sqrt{\frac{COV(X, Y)}{Var(X) \times Var(Y)}}$

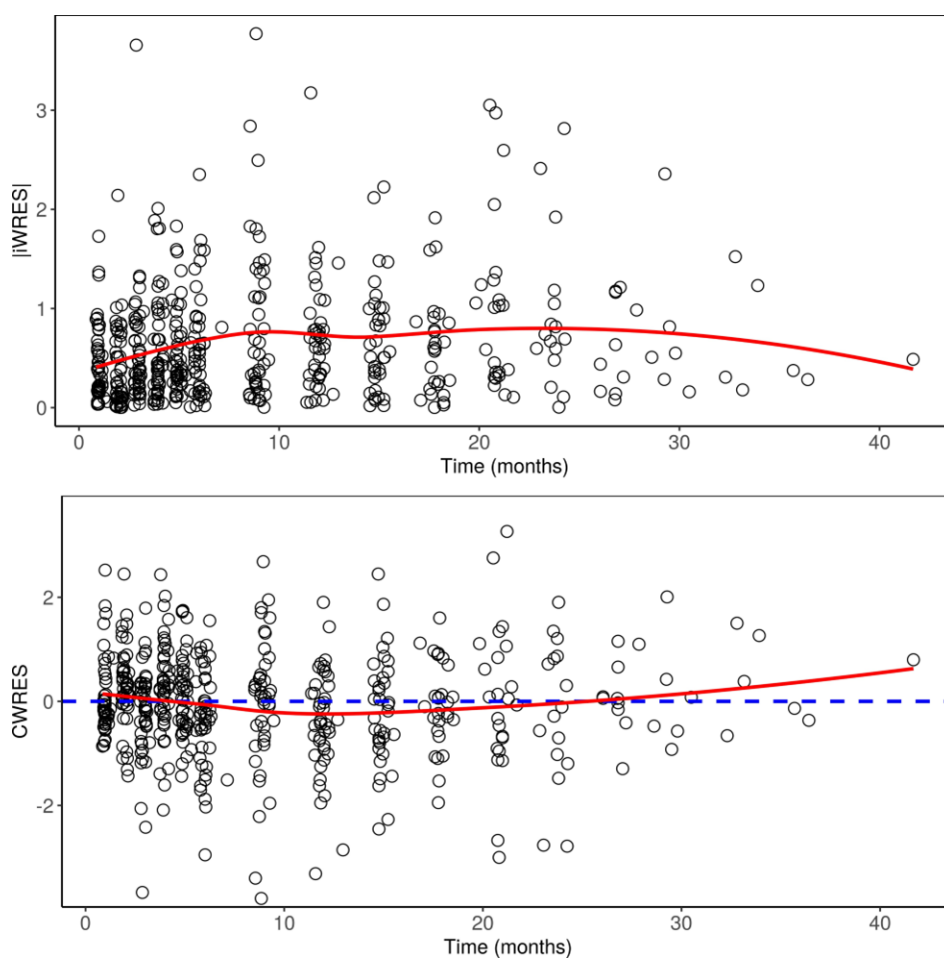
Source: Applicant. Population Pharmacodynamic Study (T067) Report.

Figure 7. HbF Observations versus Predictions



Source: Applicant. Population Pharmacodynamic Study (T067) Report.

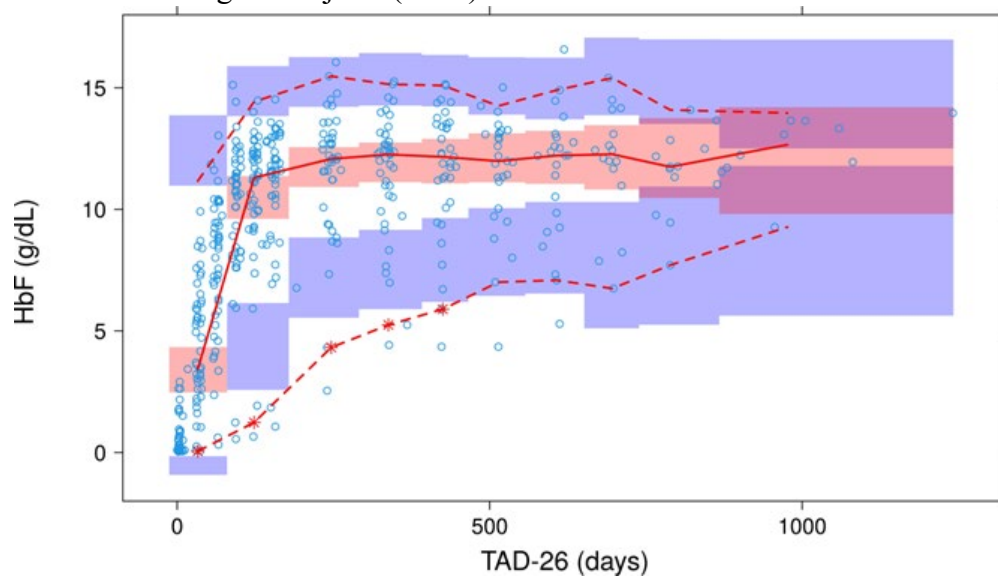
Figure 8. Individual and Conditional Weighted Residuals of Predictions Over Time



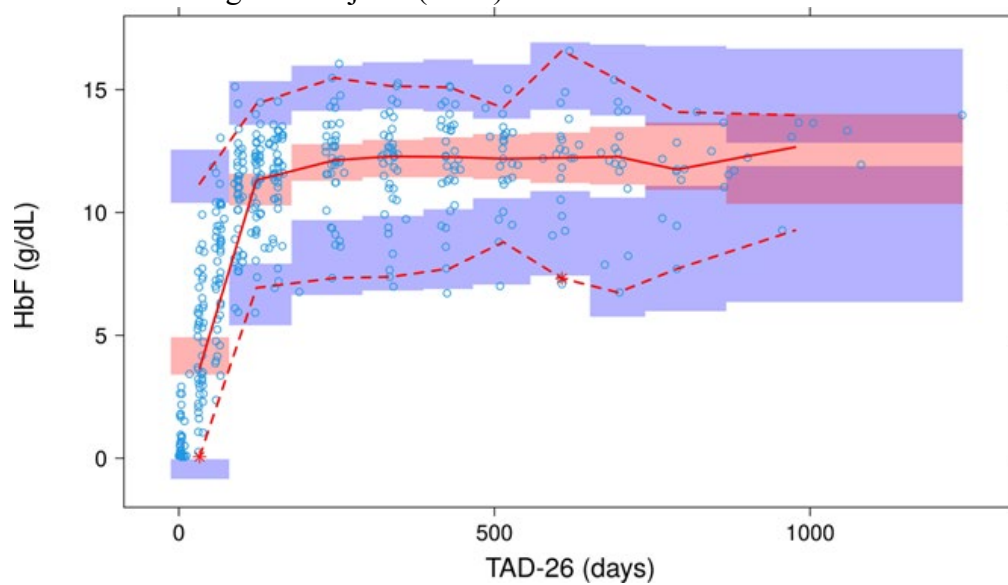
Source: Applicant. Population Pharmacodynamic Study (T067) Report.

Figure 9. Visual Predictive Check of Model

a. Including all subjects (n=48)

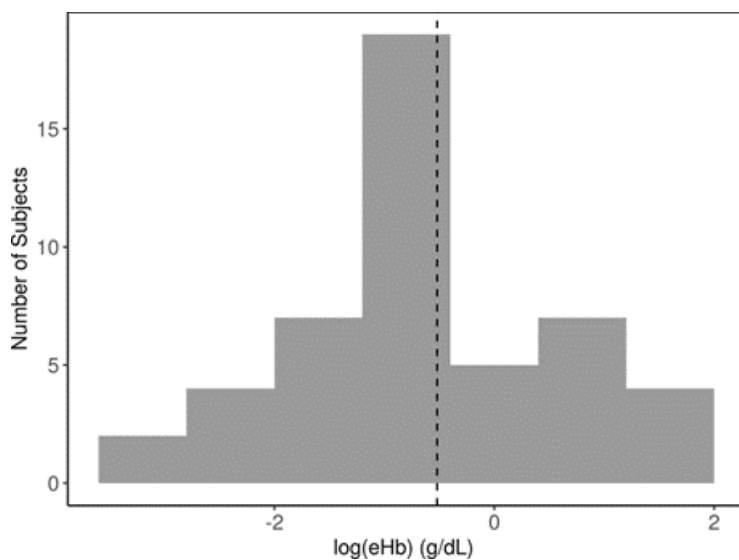


b. Excluding two subjects (n=46)



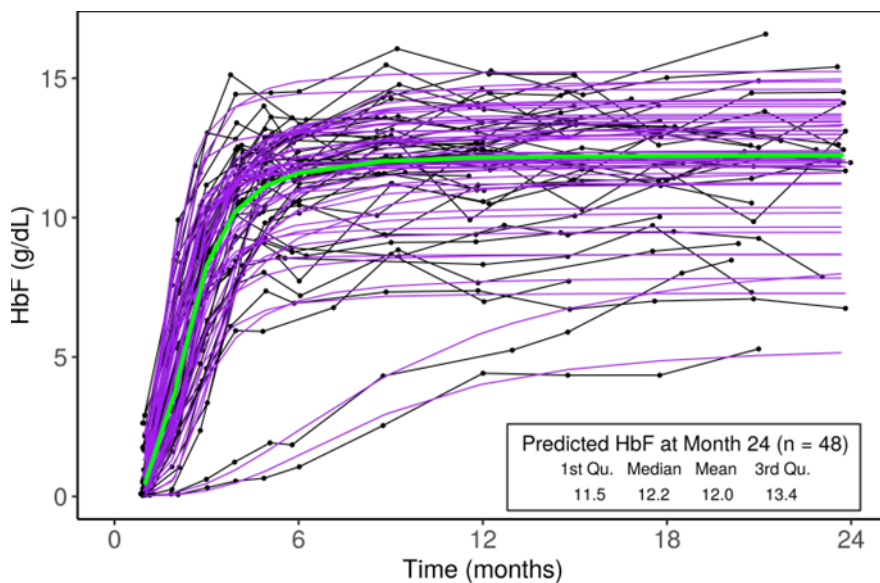
Source: Applicant. Population Pharmacodynamic Study (T067) Report.

Figure 10. Endogenous Hb Distributions



Source: Applicant. Population Pharmacodynamic Study (T067) Report.

Figure 11. Individual and Population Typical HbF Prediction to Month 24



— Observed HbF — Population Typical HbF — Individual Predicted HbF

Source: Applicant. Population Pharmacodynamic Study (T067) Report.