

**Clinical Pharmacology BLA Review**  
**Office of Clinical Evaluation (OCE)**  
**Office of Therapeutic Products (OTP)**

**Submission Number:** 125787.00

**Product Name:** Exagamglogene autotemcel (CASGEVY)

**Proposed Indication:** Treatment of sickle cell disease (SCD)

**Applicant:** Vertex Pharmaceuticals Incorporated

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## Table of Contents

1. Executive Summary.....	4
2. Recommendations .....	5
3. Background .....	5
4. Summary of Clinical Pharmacology Findings .....	7
5. Clinical Pharmacology Labeling Comments.....	12
6. Comprehensive Clinical Pharmacology Review .....	13
6.1. General Pharmacology.....	13
6.2. Pharmacokinetics/Pharmacodynamics of Busulfan.....	15
6.3. Pharmacodynamics of CASGEVY.....	18
6.3.1. Proportion of alleles with the intended genetic modification.....	18
6.3.2. Total Hemoglobin (Hb) and Fetal Hemoglobin (HbF).....	19
6.3.3. F-cells.....	20
6.4. Correlative Assessment and Population Modeling of HbF%.....	22
6.4.1. Correlation Analysis for Pharmacodynamic Parameters .....	22
6.4.2. Product Characteristics versus Clinical Outcomes.....	24
6.4.3. HbF% versus VF12.....	31
6.4.4. Population Modeling of HbF%.....	32
7. Appendix .....	34
7.1. Study#1: CTX001-121.....	34
7.2. Study#2- CTX001-131.....	37
7.3. Study #3- Population Pharmacodynamic Modeling of HbF (%).....	39

## List of Tables

Table 1: Summary of CASGEVY Dose (Study 121 PES and FAS).....	14
Table 2: Correlation Analyses for PD Parameters at Different Visits for Subjects .....	23
<b>Table 3: Summary of Correlative Assessment .....</b>	<b>27</b>
Table 4: Summary of HbF (%) and VF12 Response (Study 121 PES).....	32
Table 5: Summary of Sigmoidal Model with Offset Function .....	43

## List of Figures

Figure 1: Distribution of Individual Subject Observed Busulfan cAUC by Dosing .....	16
Figure 2: Individual Subject Time to Neutrophil Engraftment versus (A) Busulfan PK or (B) Dosing Regimen.....	17
Figure 3: Individual Subject Peripheral Blood Allelic Editing (%) Over Time (Studies 121 and 131 (FAS)).....	19
Figure 4: Summary of Total Hb (g/dL) and HbF (g/dL) Over Time (Studies 121 and 131, FAS) .....	21
Figure 5: Individual HbF (%) Over Time (Studies 121 and 131, FAS) .....	21
Figure 6 : F-cells (%) Summary Over Time (Studies 121 and 131, [SCD]FAS) .....	22
Figure 7 : Average HbF (%) Versus Average Allelic Editing (%) in Peripheral Blood .....	24
Figure 8: Average Bone Marrow Allelic Editing (%) and Average Peripheral Blood .....	28
Figure 9: VF12 Versus CASGEVY Dose ( $\times 10^6$ CD34+ cells/kg) and Allelic Editing (%) in Drug Product (Studies 121 and 131, [SCD]PES) .....	30
Figure 10: HbF% observations vs. predictions (sigmoidal model with offset function)...	44
Figure 11: Individual and conditional weighted residuals of predictions over time.....	44
Figure 12: Visual predictive check of model including offset function .....	45
Figure 13: Individual and population typical HbF% predictions to month 48.....	46

## 1. Executive Summary

CASGEVY (exagamglogene autotemcel) is a cellular gene therapy consisting of autologous CD34<sup>+</sup> HSCs edited by CRISPR/Cas9-technology at the erythroid specific enhancer region of the *BCL11A* gene to reduce BCL11A expression in erythroid lineage cells, leading to increased fetal hemoglobin (HbF) protein production.

In this BLA submission, the applicant developed CASGEVY for treatment of Sickle cell disease (SCD) in patients 12 years and older with recurrent vaso-occlusive crises (VOCs). The data supporting clinical pharmacology was obtained from a study (#CTX-001-121) and an open-label long-term follow-up study (#CTX-001-131). The primary efficacy endpoint was absence of severe vaso-occlusive crises free for at least 12 consecutive months (VF12). The clinical pharmacology review will focus on evaluation of pharmacodynamic (PD) parameters (total Hb, HbF, and F-cells). The proportion of alleles with the intended genetic modification in the peripheral blood, and CD34<sup>+</sup> cells of the bone marrow was also evaluated as part of PD assessment.

The mean proportion of alleles with the intended genetic modification in peripheral blood was generally maintained  $\geq 70\%$  from Month 2 onward, through the duration of follow-up in Studies 121 and 131. The mean (SD) proportion of total Hb comprised of HbF (%) was 36.9% (9.0%) at Month 3 and was maintained at  $\geq 40\%$  from Month 6 over the duration of follow-up. Correlative analysis demonstrates a correlation of the earlier timepoint (Month 6) with later timepoints (e.g., Month 12 & 24) for parameters such as HbF% and allelic editing in bone marrow and peripheral blood. The empirical population pharmacodynamic model reasonably described the observed HbF% vs time profile up to Month 24. No relevant dose-response relationship was identified for HbF% and clinical efficacy (VF12).

Overall, dose-response and correlative assessment did not identify CASGEVY dose as a factor affecting HbF% or clinical efficacy (VF12) based on the limited clinical data. The product allelic editing and % net increase in gamma globin expression appears to correlate with in vivo persistence of gene edited cells. However, the available data don't allow to derive a threshold of in vivo persistence that correlates with HbF (%) or VF12.

The recommended minimum single intravenous dose ( $3.0 \times 10^6$  CD34+ cells/kg) of CASGEVY for treatment of SCD is acceptable from clinical pharmacology perspective.

## 2. Recommendations

The clinical pharmacology information in this BLA is acceptable to support approval from a Clinical Pharmacology perspective. Labeling recommendations are provided in section 5.

## 3. Background

Sickle cell disease (SCD) is monogenic blood disorder affecting more than 100,000 people in the United States and approximately 20 million people worldwide. SCD is caused by a single nucleotide change in the  $\beta$ -globin gene (HBB), replacing a hydrophilic glutamic acid with a hydrophobic valine at the sixth residue. The resulting hemoglobin S (HbS) polymerizes under hypoxic or acidic conditions, deforming the red blood cells (RBCs) into a rigid sickle shape that can block blood flow. The damaged RBCs and blocked blood flow through the body can lead to serious problems, including stroke, eye problems, infections, and episodes of pain called vaso-occlusive crises (VOCs). SCD is a lifelong illness, and patients with SCD have a shorter lifespan than patients who do not have SCD<sup>1,2</sup>.

Fetal hemoglobin (HbF), consisting of two alpha and two gamma chains, is known to modulate the clinical and hematologic features of SCD. The production of HbF is developmentally regulated so that the level of  $\gamma$ -globin that is produced in utero decreases postnatally as the production of beta-globin and adult hemoglobin (consisting of two alpha

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<sup>1</sup> <https://www.nhlbi.nih.gov/health/sickle-cell-disease>

<sup>2</sup> [So Hyun Park<sup>1</sup>, Gang Bao \(2021\)](#). CRISPR/Cas9 gene editing for curing sickle cell disease. Transfus Apher Sci 60(1):103060

and two beta chains) increases<sup>3</sup>. The pathophysiology of SCD is dependent on the polymerization of deoxy sickle hemoglobin where higher levels of HbF slow down this process<sup>2, 4</sup>.

Available treatment for SCD includes hydroxyurea, L-glutamine, voxelotor, crizanlizumab, as well as allogeneic hematopoietic stem cell transplantation (HSCT), which may decrease the frequency of VOCs. Allogeneic HSCT may be curative, but availability is limited to the small minority with appropriate donors. A patient needs a well-matched donor to have the best chance for a successful transplant<sup>1,2</sup>. Thus SCD treatment constitutes an unmet need. Ex vivo genetically modified autologous hematopoietic stem and progenitor cells (HSPCs) is an approach being explored to potentially cure SCD regardless of the availability of suitable donors<sup>3</sup>.

In this BLA submission, the applicant developed CASGEVY (exagamglogene autotemcel for treatment of SCD in patients 12 years and older with recurrent VOCs. CASGEVY (formerly known as Exa-cel or CTX001) is a cellular gene therapy consisting of autologous CD34+ HSCs edited by clustered regularly interspaced short palindromic repeats (CRISPR/Cas9)-technology at the erythroid enhancer region of the B-Cell Lymphoma/Leukemia 11A (*BCL11A*) gene to reduce BCL11A expression in erythroid lineage cells, leading to increased HbF protein production. BCL11A is known to regulate HbF level and suppresses fetal hemoglobin expression by association with other DNA-bound factors at numerous positions within the beta-globin locus, including direct HBG promoter repression by BCL11A. Therefore, HbF reactivation through disruption of BCL11A is a potential target for therapeutic gene editing for the treatment of SCD<sup>2</sup>.

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<sup>3</sup> Frangoul et al. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and  $\beta$ -Thalassemia. *N Engl J Med* 384:252-260

<sup>4</sup> Akinsheye et al. (2021). Fetal hemoglobin in sickle cell anemia. *Blood*. 118(1):19-27

#### 4. Summary of Clinical Pharmacology Findings

The data supporting clinical pharmacology was obtained from a study (#CTX-001-121) and an open-label long-term follow-up study (#CTX-001-131):

- Study 121 is an ongoing global, single-arm, open-label, multi-site, single-dose, Phase 1/2/3 study in subjects aged 12 to 35 years (inclusive) who have severe SCD evaluating the safety and efficacy of a single dose of Exa-cel.
- Study 131 is an ongoing global, 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 including Study 121.

The primary efficacy endpoint was absence of severe vaso-occlusive crises (VOC) free for at least 12 consecutive months (VF12). The clinical pharmacology review focus evaluation of pharmacodynamic (PD) parameters (total Hb, HbF, and F-cells). The proportion of alleles with the intended genetic modification in the peripheral blood, and CD34+ cells of the bone marrow was also evaluated as part of PD assessment. The applicant also conducted population PD analysis of HbF to quantitatively describe the longitudinal HbF level. For initial clinical pharmacology assessment longitudinal data for subjects with SCD from Studies 121 and 131 were pooled based on the FAS (i.e., for all subjects dosed with CASGEVY; data cutoff date February 10, 2023) and for additional analysis (e.g., correlative/population PD) updated data (data cutoff date June 14, 2023) were employed. The major clinical pharmacology findings are summarized in the following sections.

##### **Pharmacokinetics (PK)**

- PK studies such as absorption, distribution, metabolism, and excretion (ADME) were not conducted for CASGEVY.
- Up to 7 blood samples were collected to determine the PK/exposure of the conditioning regimen (i.e., busulfan).

- In Study 121, 28 subjects (66.7%) and 14 subjects (33.3%) received Q6h and Qd regimen of busulfan, respectively. The mean (SD) total busulfan dose was 12.35 (2.33) mg/kg. The target busulfan cAUC was 74 mg\*h/L (range: 59 to 89) for the q6h and 82 mg\*h/L (range: 74 to 90) for the qd regimen.
- Thirty-five (83%; N = 42) subjects were within the protocol-specified busulfan cAUC target range across the q6h and qd regimens.
- Subgroup analyses of busulfan cAUC were performed by age at screening ( $\geq 12$  and  $< 18$  years of age and  $\geq 18$  and  $\leq 35$  years of age), sex, race, and body weight. No clinically relevant effects of age at screening, sex, or body weight on busulfan cAUC were observed.
- The pharmacodynamic (PD) effect of busulfan was evaluated by assessment of neutrophil and platelet engraftment following infusion of CASGEVY. PK (observed busulfan cAUC)/PD (neutrophil or platelet engraftment) analyses were performed for all subjects who received a dose of CASGEVY. No relevant relationship was observed between individual subject time to neutrophil engraftment and observed busulfan cAUC or busulfan dosing regimen (q6h or qd). A similar result was obtained for busulfan PK versus platelet engraftment.

### **Pharmacodynamics (PD)**

**Persistence of Gene Edited Cells:** Monitoring of proportion of alleles with the intended genetic modification in the peripheral blood and CD34+ cells of the bone marrow were monitored to demonstrate pharmacodynamic (PD) activity of CASGEVY.

- The product allelic editing in the drug product (mean [SD] of 89.9% [6.1%]) and range from 65 to 95 %.
- In the FAS, the mean (SD) proportion of alleles with the intended genetic modification in the CD34+ cells of the bone marrow were 86.6% (7.8%) at the first timepoint of evaluation (Month 6) and was sustained with mean  $\geq 85\%$  thereafter through the duration of follow-up in Study 121.



- The mean proportion of alleles with the intended genetic modification in peripheral blood was generally maintained  $\geq 70\%$  from Month 2 onward, through the duration of follow-up in Studies 121 and 131 in the [SCD]FAS.
- Based on the limited data, there is no major difference between adolescent vs adult subjects and male vs female for % allelic editing in peripheral blood.

Overall, gene edited cells were detected at 1 month, reach stable level within 3-6 months and persist for over 24 months following infusion of CASGEVY.

**Total hemoglobin (Hb) and fetal hemoglobin (HbF):** Subjects with SCD have endogenous  $\beta^S$ -globin expression from mutated  $\beta$ -globin gene, and treatment with CASGEVY is expected to induce reactivation of  $\gamma$ -globin resulting in increased HbF production. In the FAS, after CASGEVY infusion in Study 121, increases in mean HbF levels (absolute and %) occurred early (Month 3) and were maintained over time from approximately Month 6 onward, through the duration of follow-up (i.e., 24 Months in Study 121). The total Hb level also increases over time. The following is summary of the results total Hb and HbF%:

- The mean (SD) total Hb levels were 11.9 (1.5) g/dL at Month 3 and were maintained with mean  $\geq 11.1$  g/dL from Month 6 onward (Figure 4).
- The mean (SD) proportion of total Hb comprised of HbF (%) was 36.9% (9.0%) at Month 3 and was maintained at generally  $\geq 40\%$  from Month 6 over the duration of follow-up.
- All 30 (100%) subjects in the [SCD]PES had sustained HbF  $\geq 20\%$  for at least 12 consecutive months starting 60 days after the last RBC transfusion.
- Subgroup analysis based on age (adolescent vs adult) and sex (male vs female) showed comparable HbF %. Limited data are available for other subgroup analysis (e.g., genotypes and race).

**F-cells:** F-cells are the subpopulation of red blood cells (RBCs) that contain HbF. Measurement of proportion of F-cells (circulating RBCs expressing detectable levels of HbF) allows measurement of the distribution of HbF across the RBCs in circulation. The mean (SD) proportion of F-cells in the FAS was 20 % (13%) at baseline, 71% (14%) at Month 3, increased to 93% (13%) at Month 6, and was thereafter maintained at  $\geq 95\%$  over the duration of follow-up, which is consistent with the increases in HbF levels observed after CASGEVY infusion.

### **Durability of Pharmacodynamic Effect:**

- A positive correlation of the earlier timepoint (Month 6) with later timepoints was observed for all parameters including HbF (%) and allelic editing in bone marrow and peripheral blood. These results suggest that subjects with shorter follow-up (up to Month 6) will have similar durable PD activity (i.e., HbF%, allelic editing in CD34+ cells of bone marrow and peripheral blood) as the subjects with longer follow-up.
- Across the allelic editing levels observed after CASGEVY infusion, there is no strong correlation between average HbF (%) levels and the average allelic editing percentages in peripheral blood.
- In general, % allelic editing and HbF%, appear to remain at relatively “stable” level from Month 6 to 24.

**Population modeling of HbF:** Population pharmacodynamic (popPD) analysis of HbF% was conducted to characterize longitudinal change in HbF% and predict HbF% at Months 24 and 48 following treatments with CASGEVY.

- The empirical popPD model reasonably described the observed HbF% vs time profile up to Month 24. However, the current popPD analysis cannot be used for extrapolation of HbF% up to Months 48 due to limited data with long-term follow-up and lack of mechanistic component to describe the variability of HbF%.
- Based on simulations, the median predicted Month 24 HbF% was 42.1% (range: 25.7% to 49.9%), with 97.5% of the subjects at or above 32.1%. This model predicted HbF% at Month 24 was consistent with the observed data.
- The popPD model was also used to screen for potential impact of intrinsic factors (age, race, sex, weight), extrinsic factors (busulfan cAUC), and manufacturing attributes (administered CD34+ cells/kg, percent allelic editing in drug product) against the Empirical Bayes Estimates (EBEs) of the time to half-maximal HbF (%) and steady state HbF (%) parameters from the reported base popPD model.

- Across all the evaluations of intrinsic, extrinsic, and manufacturing factors, none of the categorical comparisons reach significance at the  $P = 0.05$  level. For the range of factors explored no clinically relevant effects of intrinsic, extrinsic, or manufacturing factors are observed.

## **Dose-Response Analysis and Correlative Assessment**

**Dose-Response:** Per Study 121 protocol, the minimum dose of CASGEVY was  $3 \times 10^6$  CD34+ cells/kg which was derived based on clinical experience from autologous transplantation of CD34+ cells. The protocol specified maximum cell dose of  $20 \times 10^6$  CD34+ cells/kg based on manufacturing capabilities.

- A total of 44 subjects (full analysis sets, FAS) received CASGEVY infusion, with a median (range) dose of  $4 \times 10^6$  (2.9 to  $14.4 \times 10^6$ ) CD34+ cells/kg.
- Three subjects received doses of  $2.9 \times 10^6$  CD34+ cells/kg which was slightly below the protocol specified range.
- There was a trend for positive correlation between average allelic editing in bone marrow and average allelic editing in drug product (Pearson correlation = 0.87) and a similar trend between allelic editing in peripheral blood and allelic editing in drug product (Pearson correlation = 0.67).
- A trend for negative correlations were observed between the CASGEVY dose and the average allelic editing in bone marrow and between CASGEVY dose and the average allelic editing in peripheral blood. All doses resulted in allelic editing  $\geq 50\%$  in both bone marrow and peripheral blood across the dose range administered.
- A positive trend was noted for correlation of % net increase in gamma globin vs HbF% or allelic editing in bone marrow.
- The Pearson correlations for all other analyses were  $<0.24$ , indicating no strong correlation between drug characteristics (e.g. CASGEVY dose and allelic editing in drug product) and PD parameters (e.g. HbF(%), time to neutrophil engraftment, and time to platelet engraftment).
- Subjects achieved VF12 regardless of CASGEVY dose or percent allelic editing in drug product. The CASGEVY dose administered in the subject who did not achieve

VF12 largely overlapped with the CASGEVY doses administered to the subjects who did achieve VF12. Similarly, allelic editing in drug product for the subject who did not achieve VF12 largely overlapped with the allelic editing in drug product for the subjects who did achieve VF12.

- In three subjects that received below the protocol recommended dose (i.e.,  $2.9 \times 10^6$  CD34+ cells/kg), no difference was noted in any of the evaluated clinical outcomes, including allelic editing in bone marrow and peripheral blood, HbF (%), VF12, and time to platelet and neutrophil engraftment as compared with subjects who received the recommended dose of CASGEVY drug product ( $\geq 3 \times 10^6$  CD34+ cells/kg).

Overall, dose-response and correlative assessment did not identify CASGEVY dose as a primary factor affecting HbF or clinical efficacy (VF12) based on the current limited clinical data. The product allelic editing and percent net increase in gamma globin expression appears to correlate with in vivo persistence of gene edited cells. However, the available limited data don't allow to derive a threshold of in vivo persistence that correlates with HbF (%) or VF12.

## 5. Clinical Pharmacology Labeling Comments

At the time of finalization of this review memorandum, negotiations on the labeling are ongoing between FDA and the Applicant. As they currently stand, the following are clinical pharmacology labeling comments that will be communicated with the Applicant:

### 12.1. Mechanism of Action

- Recommended revision to provide more specific information based on mechanistic and pharmacodynamic data

### 12.2. Pharmacodynamics

- Recommended to move the HbF% information presented in section 14 to section 12.2.

- Recommended to summarize the results of the proportion of allelic edited cells in peripheral blood and bone marrow and to summarize the results of subgroup analysis including intrinsic and extrinsic factors for allelic editing and HbF%.

## 6. Comprehensive Clinical Pharmacology Review

### 6.1. General Pharmacology

CASGEVY is a cellular gene therapy consisting of autologous CD34<sup>+</sup> HSCs edited by CRISPR/Cas9-technology at the erythroid specific enhancer region of the *BCL11A* gene to reduce BCL11A expression in erythroid lineage cells. The reduction of *BCL11A* gene transcription leads to increases in levels of HbF.

After CASGEVY infusion, the edited CD34<sup>+</sup> cells engraft in the bone marrow and differentiate to erythroid lineage cells with reduced BCL11A expression. Reduced BCL11A expression results in an increase in  $\gamma$ -globin expression and HbF protein production in erythroid cells. In patients with severe sickle cell disease, HbF expression reduces intracellular hemoglobin S (HbS) concentration, preventing the red blood cells from sickling and addressing the underlying cause of disease, thereby eliminating VOCs. Following successful engraftment, the effects of CASGEVY are expected to be over 24 Months based on the persistence of proportion of allelic edited CD34 cells and HbF expression.

Myeloablation is a required step before CASGEVY infusion to deplete endogenous HSPCs from the subject's bone marrow and allow hematopoietic repopulation with gene edited CD34<sup>+</sup> human HSPCs. A single agent busulfan myeloablative conditioning was used in Study 121. Busulfan was administered intravenously through a central venous catheter daily at a starting dose of 3.2 mg/kg/day for 4 consecutive days. Once daily (qd) dosing was the preferred schedule, but the busulfan dose regimen could be adjusted to be given every 6 hours (q6h) per site's standard practice. Busulfan's effectiveness in

myeloablation is dependent on cumulative exposure (cumulative area under the concentration versus time curve, cAUC) over 4 days of dosing.

Per Study 121 protocol, the minimum dose of CASGEVY was  $3 \times 10^6$  CD34+ cells/kg which was derived based on clinical experience from autologous transplantation of CD34+cells. The protocol specified maximum cell dose of  $20 \times 10^6$  CD34+ cells/kg based on manufacturing capabilities. A total of 42 subjects (full analysis sets, FAS) received CASGEVY infusion, with a median (range) dose of  $4.1 \times 10^6$  (2.9 to 14.4) CD34+ cells/kg. A total of 20 subjects were included in the primary efficacy sets (PES) and received median (range) CASGEVY dose of  $3.6 \times 10^6$  (2.9 to 14.4) CD34+ cells/kg. At the 90-day safety update (June 14, 2023), two additional subjects were dosed with CASGEVY resulting in a total of 44 subjects (FAS). The PES was also increased by 10 subjects resulting in a total of 30 subjects for PES (Table 1). Three subjects received doses of  $2.9 \times 10^6$  CD34+ cells/kg which was below the protocol specified range. In the 3 subjects who received an infusion of  $2.9 \times 10^6$  CD34+ cells/kg, clinical pharmacology assessment was comparable between those who received doses of below or higher than  $3 \times 10^6$  CD34+ cells/kg. The recommended dose of CASGEVY is a single intravenous dose of at least  $3.0 \times 10^6$  CD34+ cells/kg.

**Table 1: Summary of CASGEVY Dose (Study 121 PES and FAS)**

Parameter	Initial BLA (Study 121)		BLA Day 90 Update (Study 121)	
	PES N = 20	FAS N = 42	PES N = 30	FAS N = 44
<b>CASGEVY dose (<math>10^6</math> CD34+ cells/kg)</b>				
n	20	42	30	44
Mean (SD)	4.8 (3.35)	4.8 (2.49)	4.8 (2.82)	4.7 (2.45)
Median	3.6	4.1	4.0	4.0
Min, max	2.9, 14.4	2.9, 14.4	2.9, 14.4	2.9, 14.4

Source: Table 1; Exac-cel SCD clinical overview addendum

## 6.2. Pharmacokinetics/Pharmacodynamics of Busulfan

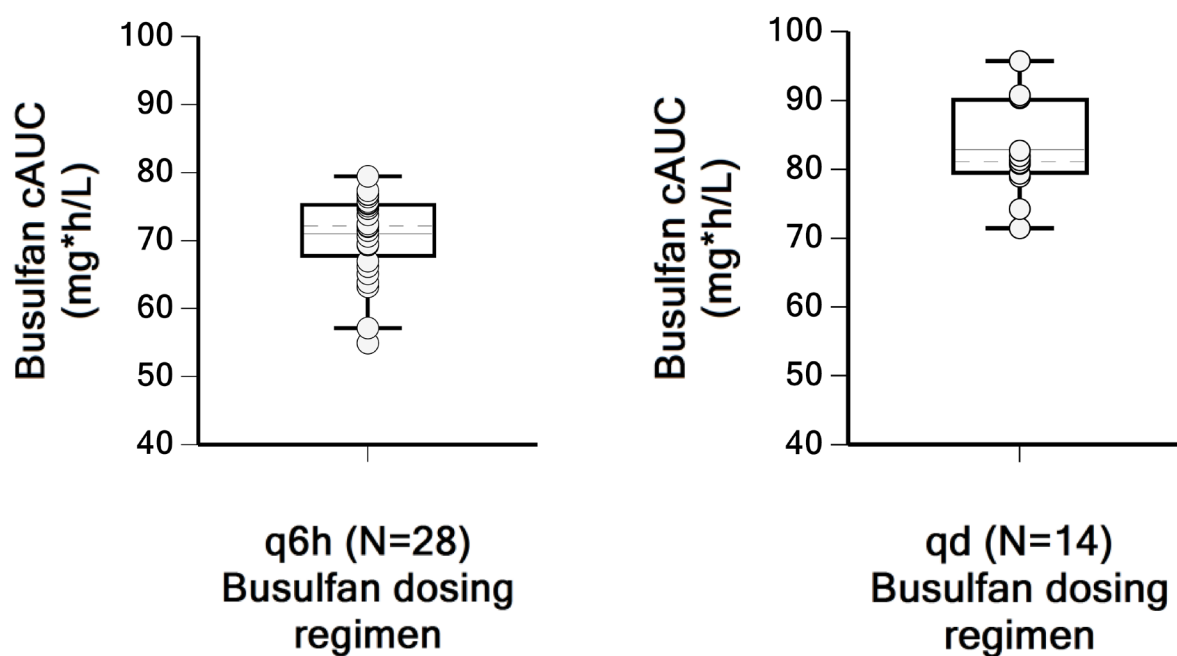
Blood samples (~7 samples within 6-8 hours of dosing) were collected on Days 1 and 3 of busulfan dosing to determine pharmacokinetic (PK) parameters such as cAUC. For the 42 subjects in FAS, 28 subjects (66.7%) and 14 subjects (33.3%) received Q6h and Qd regimen of busulfan, respectively (Study 121). The mean (SD) total busulfan dose was 12.35 (2.33) mg/kg. The cumulative busulfan exposure (cAUC) is presented in Figure 1. Regardless of age group, the target busulfan cAUC was 74 mg\*h/L (range: 59 to 89) for the q6h and 82 mg\*h/L (range: 74 to 90) for the qd regimen. Thirty-five (83%; N = 42) subjects were within the protocol-specified busulfan cAUC target range across the q6h and qd regimens. Twenty-six (93%; N = 28) subjects receiving the q6h regimen and 9 (64%; N = 14) subjects receiving the qd regimen were within the protocol specified cAUC target range. With the q6h regimen, 2 subjects had exposure below the target range. With the qd regimen, 1 subject had an exposure below the target range and 4 subjects had exposures above the target range.

Subgroup analyses of busulfan cAUC were performed by age at screening ( $\geq 12$  and  $< 18$  years of age and  $\geq 18$  and  $\leq 35$  years of age), sex, race, and body weight. No clinically relevant effects of age at screening, sex, or body weight on busulfan cAUC were observed. However, it is important to note that most subjects (85.7%) were Black or African American and it is difficult to evaluate the impact of race on PK/PD of busulfan or CASGEVY.

The pharmacodynamic (PD) effect of busulfan was evaluated by assessment of neutrophil and platelet engraftment following infusion of CASGEVY. PK (observed busulfan cAUC)/PD (neutrophil or platelet engraftment) analyses were performed for all subjects who received a dose of CASGEVY. Time to neutrophil engraftment was defined as the first day of three consecutive measurements with ANC  $\geq 500/\mu\text{L}$  on three different days. The median (range) time from the day of CASGEVY infusion to neutrophil engraftment was 27 (15 to 40) days. No relevant relationship was observed between individual subject time to neutrophil engraftment and observed busulfan cAUC or busulfan

dosing regimen (q6h or qd) (Figure 2). A similar result was obtained for busulfan PK versus platelet engraftment.

**Figure 1: Distribution of Individual Subject Observed Busulfan cAUC by Dosing Regimen**



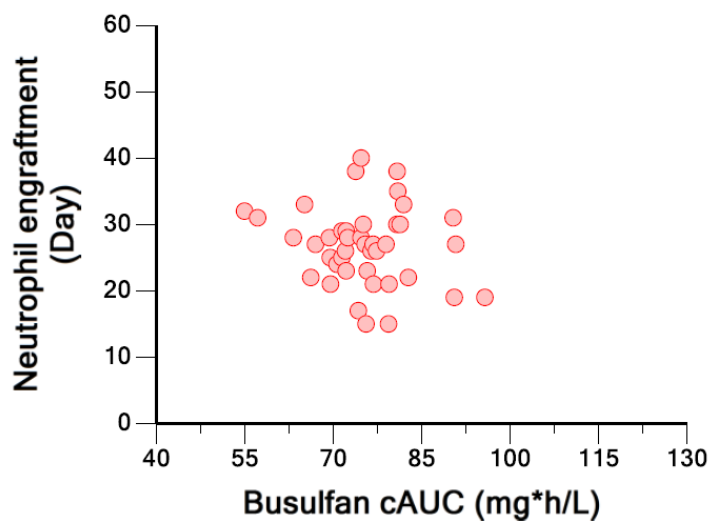
Source: Figure 14.4.2.1; CSR (#Study 121)

Note: Solid lines represent the mean; dotted lines represent the median; box represents the IQR; whiskers represent the observed minimum and maximum up to  $\pm 1.5 \times \text{IQR}$ , and symbols represent individual subjects busulfan cAUC.

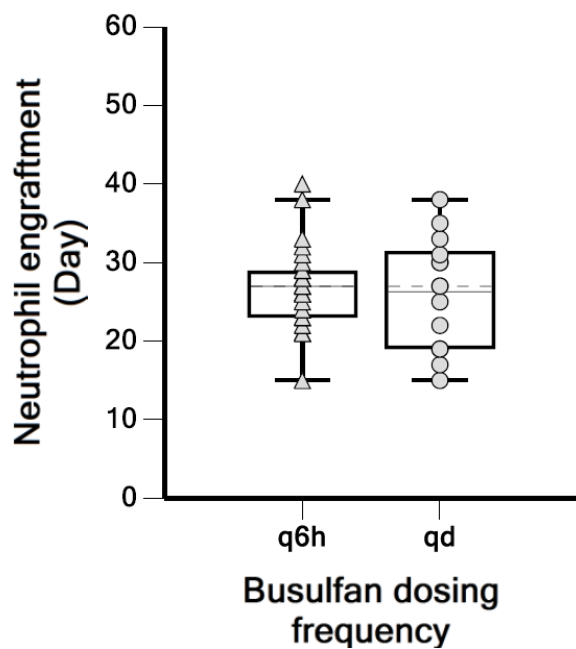


**Figure 2: Individual Subject Time to Neutrophil Engraftment versus (A) Busulfan PK or (B) Dosing Regimen**

**(A)**



**(B)**



Source: Figure 14.4.2.2 & 14.4.2.3; CSR (#Study CTX001-121)

### 6.3. Pharmacodynamics of CASGEVY

#### 6.3.1. Proportion of alleles with the intended genetic modification

Gene edited CD34+ HSPCs is expected to undergo self-renewal and establish a population of modified erythroid lineage cells, which pass the transgene to daughter blood cells on differentiation. Thus, monitoring of proportion of alleles with the intended genetic modification in the peripheral blood and CD34+ cells of the bone marrow reflect pharmacodynamic (PD) activity of CASGEVY.

The proportion of allelic editing in the product was initially characterized prior to product administration. The product allelic editing in the drug product (mean [SD] of 89.9% [6.1%]) and range from 65 to 95 %. There is no major difference in allelic editing between the two genotypes (Non-Beta S/Beta S (n=4) vs Beta S/Beta S (n=38), adolescent (n=12) vs adult (n=30) subjects and male (n=24) vs female (n=18).

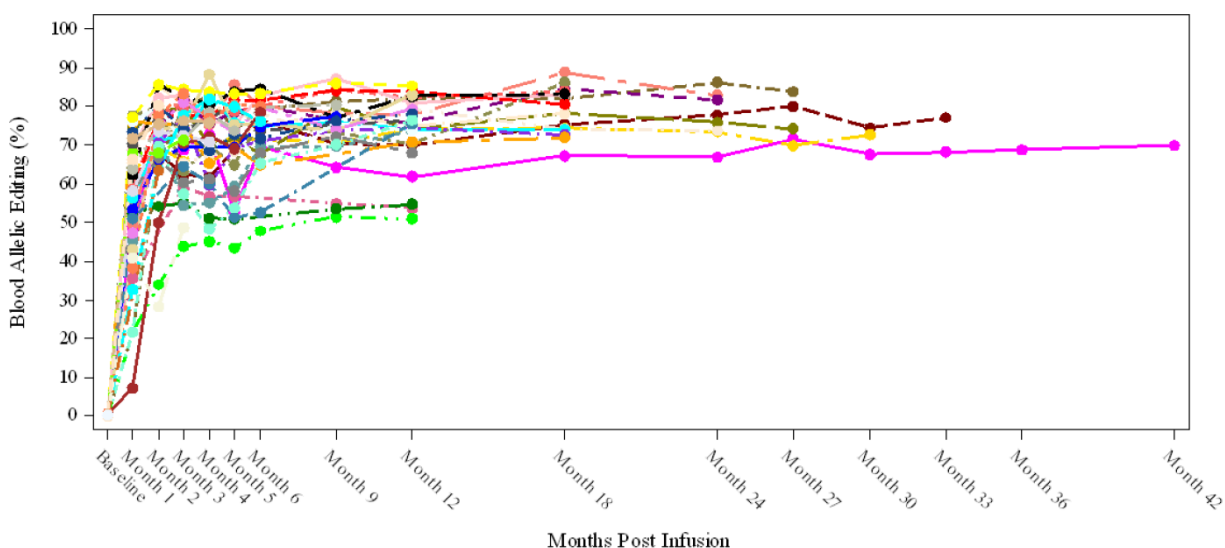
In the FAS, the mean (SD) proportion of alleles with the intended genetic modification in the CD34+ cells of the bone marrow were 86.6% (7.8%) at the first timepoint of evaluation (Month 6) and was sustained with mean  $\geq 85\%$  thereafter through the duration of follow-up in Study 121. The bone marrow assessment was no longer collected in Study 131 for subjects with SCD. The allelic editing outcome in the CD34+ cells of the bone marrow was consistent with allelic editing in the drug product (mean [SD] of 89.9% [6.1%]). In Study 121, allelic editing was assessed more frequently in peripheral blood than in bone marrow, enabling analysis at additional time points. Peripheral blood allelic editing was also evaluated in Study 131. The mean proportion of alleles with the intended genetic modification in peripheral blood was generally maintained  $\geq 70\%$  from Month 2 onward, through the duration of follow-up in Studies 121 and 131 in the [SCD]FAS (Figure 3).

The allelic editing in the peripheral blood is lower than allelic editing in the CD34+ cells of the bone marrow because the peripheral blood samples include lymphocytes that are not derived from the edited CD34+ cells. Lymphocytes may not be completely depleted with the single agent busulfan conditioning. This results in a proportion of peripheral blood

lymphocytes having been derived prior to treatment with CASGEVY that were not edited and can lead to the observed decreased allelic editing in the peripheral blood compared to the bone marrow CD34+ cells. There is no major difference between adolescent vs adult subjects and male vs female for % allelic editing in peripheral blood.

**Reviewer comments: The in vivo allelic editing in peripheral blood and bone marrow reflect the engraftment and persistence of gene corrected cells. Edited cells in peripheral blood were detected at 1 Month, reach stable level within 3-6 and persist over 24 Months.**

**Figure 3: Individual Subject Peripheral Blood Allelic Editing (%) Over Time (Studies 121 and 131 (FAS))**



Source: Figure 11-20; CSR Study 131

### 6.3.2. Total Hemoglobin (Hb) and Fetal Hemoglobin (HbF)

Subjects with SCD have endogenous  $\beta^S$ -globin expression from mutated  $\beta$ -globin gene, and treatment with CASGEVY is expected to induce reactivation of  $\gamma$ -globin resulting in

increased HbF production. After CASGEVY infusion, subjects initially received RBC transfusion per standard practice following HSCT while HbF levels were rising, and these transfusions contribute to measurements of HbF and total Hb. Subsequently, 60 days after the last RBC transfusion (a washout period based on the half-life of a circulating transfused RBC) total Hb and HbF concentrations, and the proportion of total Hb comprised by HbF (HbF %), were considered attributable to CASGEVY treatment.

In the FAS, after CASGEVY infusion in Study 121, increases in mean HbF levels (absolute and percent occurred at Month 3) and were maintained over time from approximately Month 6 onward, through the duration of follow-up (i.e., 24 Months in Study 121). The total Hb level also increases over time (Figure 4). The following is summary of the results total Hb and HbF%:

- The mean (SD) total Hb levels were 11.9 (1.5) g/dL at Month 3 and were maintained with mean  $\geq 11.1$  g/dL from Month 6 onward (Figure 4).
- The mean (SD) proportion of total Hb comprised of HbF (%) was 36.9% (9.0%) at Month 3 and was maintained at generally  $\geq 40\%$  from Month 6 over the duration of follow-up (Figure 4).
- Individual subject HbF (%) were stable from Month 6 over time (Figure 5).
- All 30 (100%) subjects in the [SCD]PES had sustained HbF  $\geq 20\%$  for at least 12 consecutive months starting 60 days after the last RBC transfusion.
- Subgroup analysis based on age (adolescent vs adult) and sex (male vs female), showed comparable HbF %. Limited data are available for other subgroup analysis (e.g., genotypes and race).

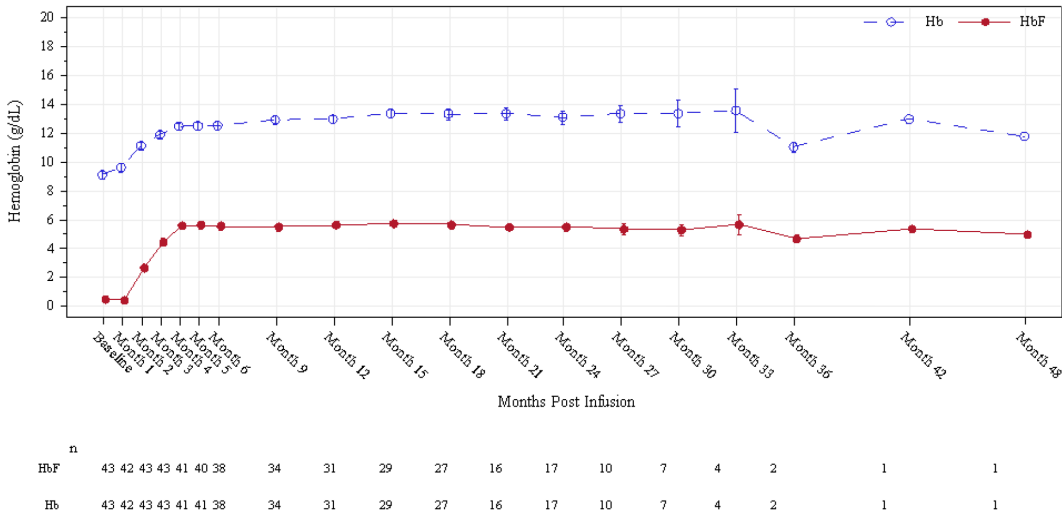
### 6.3.3. F-cells

F-cells are the subpopulation of red blood cells (RBCs) that contain HbF. Measurement of proportion of F-cells (circulating RBCs expressing detectable levels of HbF) allows measurement of the distribution of HbF across the RBCs in circulation. The mean (SD) proportion of F-cells in the FAS was 20 % (13%) at baseline, 71% (14%) at Month 3, increased to 93% (13%) at Month 6, and was thereafter maintained at  $\geq 95\%$  over the duration of follow-up (Figure 6). This profile of F-cells appears consistent with the

increases in HbF levels observed after CASGEVY infusion, suggesting pancellular distribution of HbF.

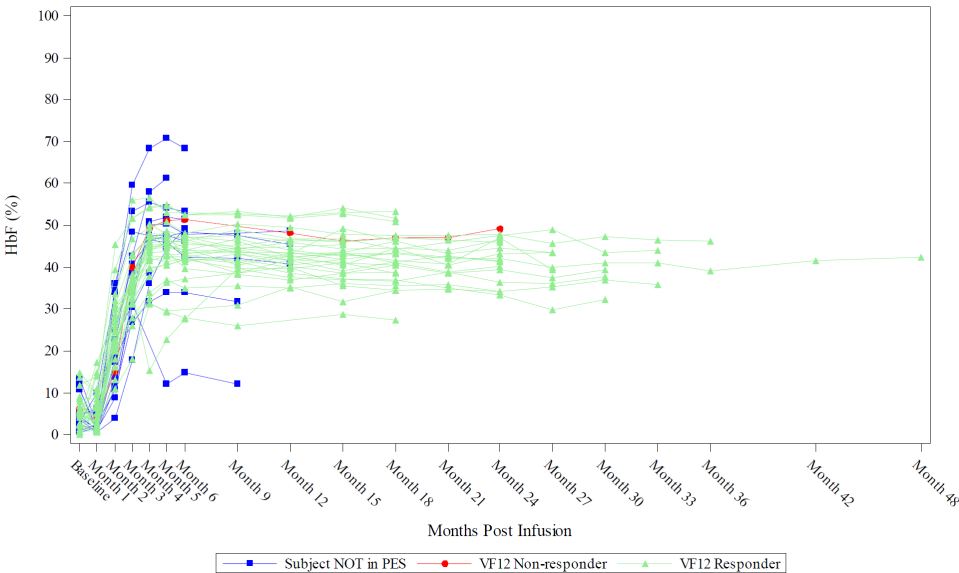
Reviewer comments: There is positive correlation between F-cells and HbF% at Month 6 following treatment with CASGEVY (correlation=0.69).

Figure 4: Summary of Total Hb (g/dL) and HbF (g/dL) Over Time (Studies 121 and 131, FAS)



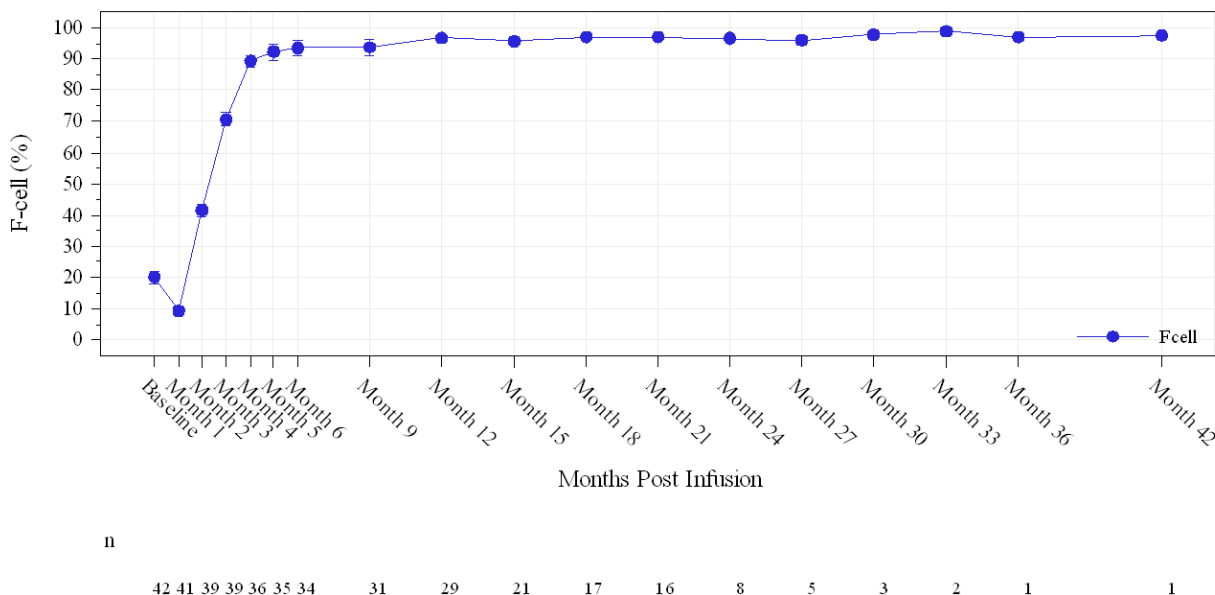
Source: Figure 14.2.6.2b(updated June 14, 2023); CSR (#Study CTX001-131)

Figure 5: Individual HbF (%) Over Time (Studies 121 and 131, FAS)



Source: Ad hoc Figure 14.2.3.3b(updated June 14, 2023); CSR (#Study CTX001-131)

**Figure 6 : F-cells (%) Summary Over Time (Studies 121 and 131, [SCD]FAS)**



**Source:** Figure 14.2.12.2b; CSR (#Study CTX001-131)

#### 6.4. Correlative Assessment and Population Modeling of HbF%

##### 6.4.1. Correlation Analysis for Pharmacodynamic Parameters

Analyses were conducted to evaluate the correlation for 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 PES to predict durable PD of subjects who are not in the PES. Data at the earlier timepoint (Month 6) for all parameters correlated with the later timepoints (Months 12, 18, 21, 24), with correlation coefficient >0.5 (Table 2). A strong correlation of the earlier timepoint (Month 6) with later timepoints was observed for all parameters including HbF (%) and allelic editing in bone marrow and peripheral blood. These results suggest that subjects with shorter follow-up (up to Month 6) will have similar durable PD activity (i.e., HbF%, allelic editing in CD34+ cells of bone marrow and peripheral blood) as the subjects with longer follow-up.

Additional correlative analysis between HbF% versus allelic editing in peripheral blood was conducted. Across the allelic editing levels observed after CASGEVY infusion, there is no strong correlation between average HbF (%) levels and the average allelic editing percentages in peripheral blood (correlation coefficient 0.11; n = 40; Figure 7). In general, % allelic editing and HbF%, appear to remain at relatively “stable” level from Month 6 to 24.

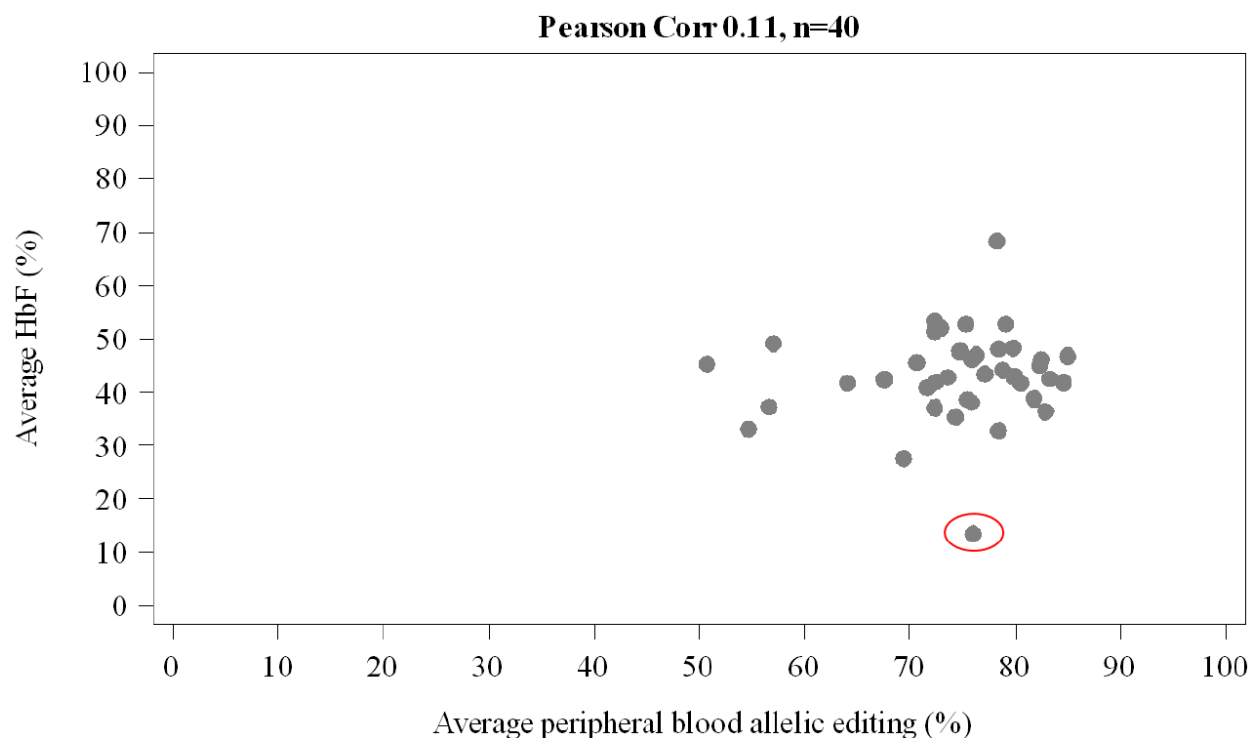
**Reviewer comment: FDA analysis using Month 6 data for HbF% and allelic editing in peripheral blood also showed no strong correlation (correlation coefficient 0.14) but both HbF% and allelic editing in peripheral blood remain at higher level from Month 6 to 24 (Figure 3& 5).**

**Table 2: Correlation Analyses for PD Parameters at Different Visits for Subjects From Study 121 (Studies 121 and 131, [SCD]PES)**

Parameter Correlation at Different Visits Statistic	Month 12	Month 18	Month 21	Month 24
<b>HbF (%)</b>				
Pearson correlation between Month 6 Visit and a specific visit				
n	20	17	15	8
Correlation	0.69	0.69	0.71	0.77
<b>Proportion of alleles with intended genetic modification in CD34<sup>+</sup> cells of bone marrow (%)</b>				
Pearson correlation between Month 6 Visit and a specific visit				
n	20	--	--	8
Correlation	0.87	--	--	0.55
<b>Proportion of alleles with intended genetic modification in peripheral blood (%)</b>				
Pearson correlation between Month 6 Visit and a specific visit				
n	19	17	--	8
Correlation	0.88	0.71	--	0.74

**Source:** Table 14.2.24b; CSR (#Study CTX001-131)

**Figure 7 : Average HbF (%) Versus Average Allelic Editing (%) in Peripheral Blood  
(Studies 121 and 131, [SCD]FAS)**



Notes: The average HbF (%) and the average peripheral blood allelic editing (%) were calculated using records from analysis visit starting from Month 6, or 60 days after the last RBC transfusion in the period of initial RBC transfusions for posttransplant support or SCD management, whichever was later.

Subject circled in red died due to COVID-19 infection.

Source: Figure 7; Response to Clin Pharm IR#1

#### 6.4.2. Product Characteristics versus Clinical Outcomes

Reviewer comments: The correlative analysis below was preformed upon FDA clinical pharmacology information request. These correlative analyses were based on the updated data (data cutoff date: June 14, 2023, following 90 days safety updated from Studies 121 and 131).

Correlative analyses between the CASGEVY product characteristics (CASGEVY dose [CD34+ cells/kg] and drug product allelic editing) and clinical outcomes including allelic editing in bone marrow CD34+ cells and peripheral blood, HbF (%), VF12 (primary



efficacy endpoint, defined as absence of severe vaso-occlusive crisis [VOC] for at least 12 consecutive months), and time to platelet and neutrophil engraftment (safety endpoints) were conducted. All analysis were conducted using the FAS (N = 44) for all clinical outcomes except VF12, which was conducted using the PES (N = 30). A summary of the analyses is provided in Table 3 and selected results are displayed in Figure 8 & 9.

There was a strong positive correlation between average allelic editing in bone marrow and average allelic editing in drug product (Pearson correlation = 0.87; Figure 8a) and a strong correlation between allelic editing in peripheral blood and allelic editing in drug product (Pearson correlation = 0.67; Figure 8b). Moderate negative correlations were observed between the CASGEVY dose and the average allelic editing in bone marrow and between CASGEVY dose and the average allelic editing in peripheral blood (Table 3). All doses resulted in allelic editing  $\geq 50\%$  in both bone marrow and peripheral blood across the dose range administered. The Pearson correlations for all other analyses were  $< 0.24$ , indicating no strong correlation between drug characteristics (CASGEVY dose and allelic editing in drug product) and HbF(%), time to neutrophil engraftment, and time to platelet engraftment (Table 3).

Subjects achieved VF12 regardless of CASGEVY dose or percent allelic editing in drug product (Figure 9). The CASGEVY dose administered in the subject who did not achieve VF12 largely overlapped with the CASGEVY doses administered to the subjects who did achieve VF12 (Figure 9a). Similarly, allelic editing in drug product for the subject who did not achieve VF12 largely overlapped with the allelic editing in drug product for the subjects who did achieve VF12 (Figure 4b). In three subjects that received below the protocol recommended dose (i.e.,  $2.9 \times 10^6$  CD34+ cells/kg), no difference was noted in any of the evaluated clinical outcomes, including allelic editing in bone marrow and peripheral blood, HbF (%), VF12, and time to platelet and neutrophil engraftment as compared with subjects who received the recommended dose of CASGEVY drug product ( $\geq 3 \times 10^6$  CD34+ cells/kg).

Reviewer comment: The correlative assessment did not identify CASGEVY dose as a factor affecting PD or clinical efficacy (VF12). The product allelic editing appears to correlate with in vivo persistence of gene edited cells. However, the available data don't

allow to derive a threshold of in vivo persistence that correlates to HbF (%) or VF12. Thus, the clinical significance of in vivo persistence vs product allelic editing remains unknown. We further explored the correlation between pharmacodynamics and engraftment parameters (HbF %, time of neutrophile or platelet engraftment, allelic editing in peripheral blood or bone marrow) with the following product characteristics:

- (b) (4)
- Total dose
- Product on-target editing frequency (%)
- (b) (4)
- (b) (4)

The FDA exploratory correlative analysis focused on the clinical parameters obtained specifically at 6 months following CASGEVY treatment with inclusion of additional product characteristics. For this exploratory analysis the correlation was considered significant when both Pearson and Spearman correlation coefficients are  $\geq 0.25$  and  $p < 0.05$ . The following is summary of FDA correlative analysis:

- A negative trend was noted for correlation of total cell dose vs allelic editing in peripheral blood (Pearson = -0.33, Spearman = -0.33)
- A positive trend was noted for correlation of product editing frequency (%) vs HbF% (Pearson = 0.28, Spearman = 0.33)
- A positive trend was noted for correlation of product editing frequency (%) vs allelic editing in peripheral blood (Pearson = 0.47, Spearman = 0.35) and product editing frequency (%) vs allelic editing in bone marrow (Pearson = 0.76, Spearman = 0.68).
- A positive trend was noted for correlation of (b) (4) vs HbF% (Pearson = 0.49, Spearman = 0.52).
- A positive trend noted for correlation of (b) (4) vs allelic editing in bone marrow (Pearson = 0.41, Spearman = 0.41).
- The correlations analyses for other product characteristics vs pharmacodynamic/engraftment parameters were not statistically significant.

**Table 3: Summary of Correlative Assessment**

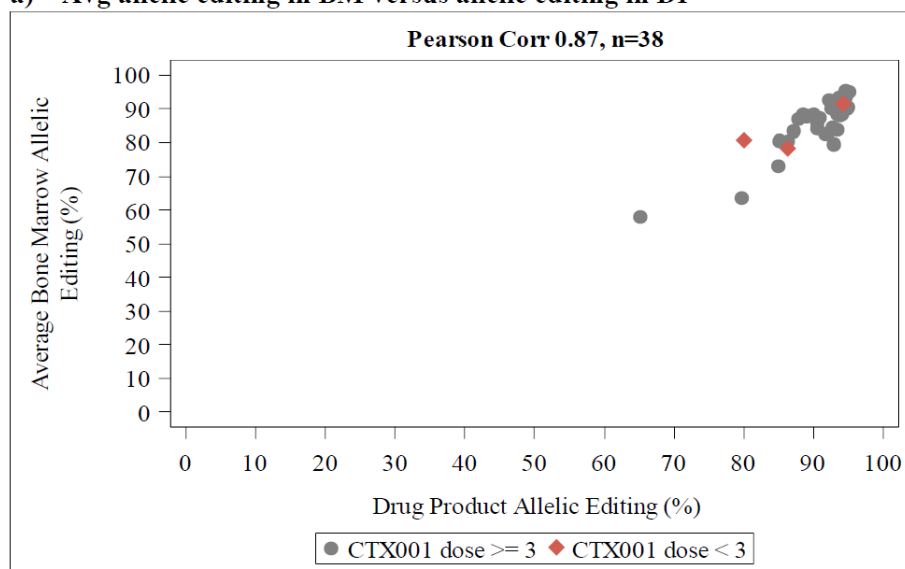
<b>Correlative analysis</b>	<b>Sample size</b>	<b>Pearson correlation</b>
Allelic editing in bone marrow vs allelic editing in drug product	38	0.87
Allelic editing in peripheral blood vs allelic editing in drug product	40	0.67
Allelic editing in bone marrow vs. CASGEVY dose	38	-0.46
Allelic editing in peripheral blood vs. CASGEVY dose	40	-0.41
HbF (%) vs CASGEVY dose	40	0.13
HbF (%) vs. allelic editing in drug product	40	0.23
Time to neutrophil engraftment vs CASGEVY dose	44	0.11
Time to neutrophil engraftment vs allelic editing in drug product	44	-0.05
Time to platelet engraftment vs CASGEVY dose	43	0.01
Time to platelet engraftment vs allelic editing in drug product	43	0.16

Note: The average HbF (%), average bone marrow and peripheral blood allelic editing (%) were calculated using records from analysis visit starting from Month 6, or 60 days after the last RBC transfusion in the period of initial RBC transfusions for posttransplant support or SCD management, whichever was later.

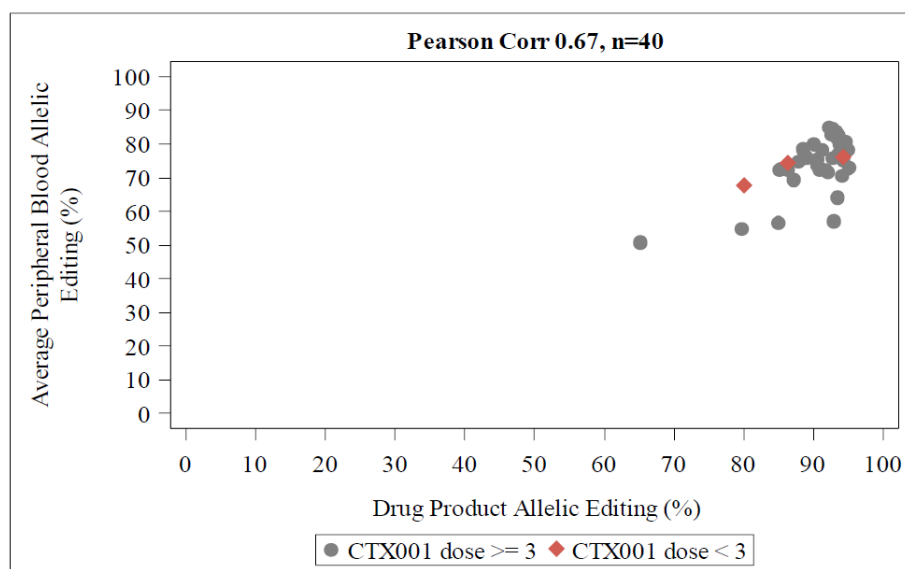
Source: Compiled from Table 1, Figure 1-7; Response to Clin Pharm IR#1

**Figure 8: Average Bone Marrow Allelic Editing (%) and Average Peripheral Blood Allelic Editing (%) versus Allelic Editing (%) in Drug Product (Studies 121 and 131, [SCD]FAS)**

**a) Avg allelic editing in BM versus allelic editing in DP**

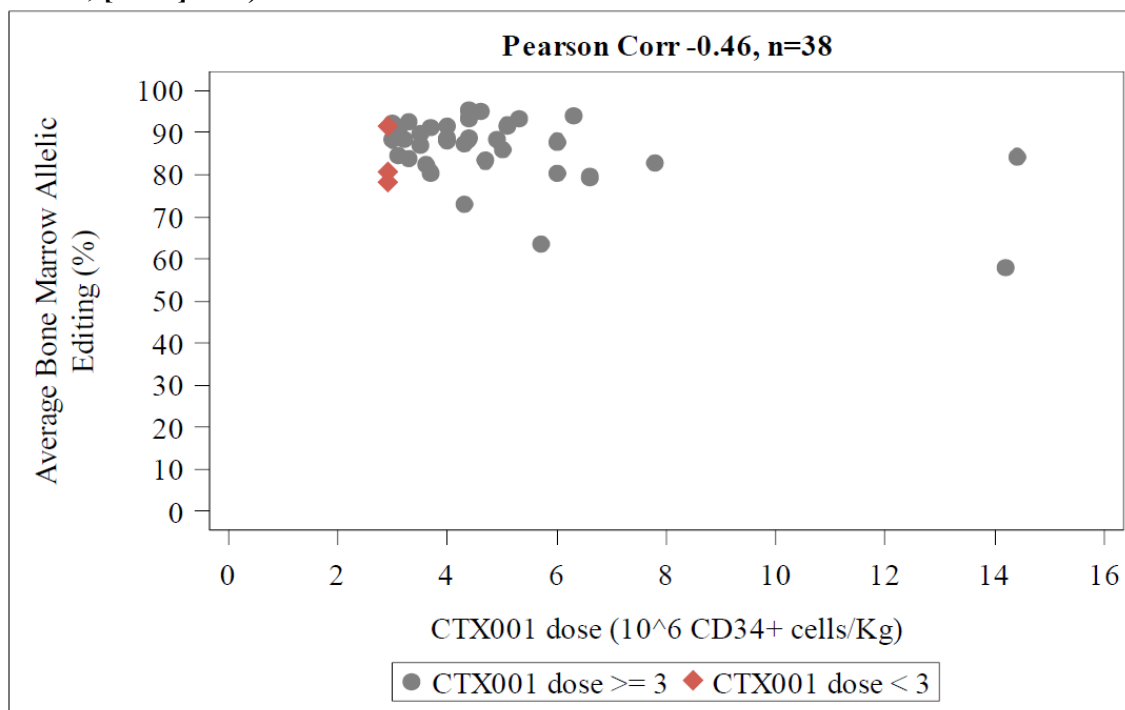


**b) Avg allelic editing in PB versus allelic editing in DP**

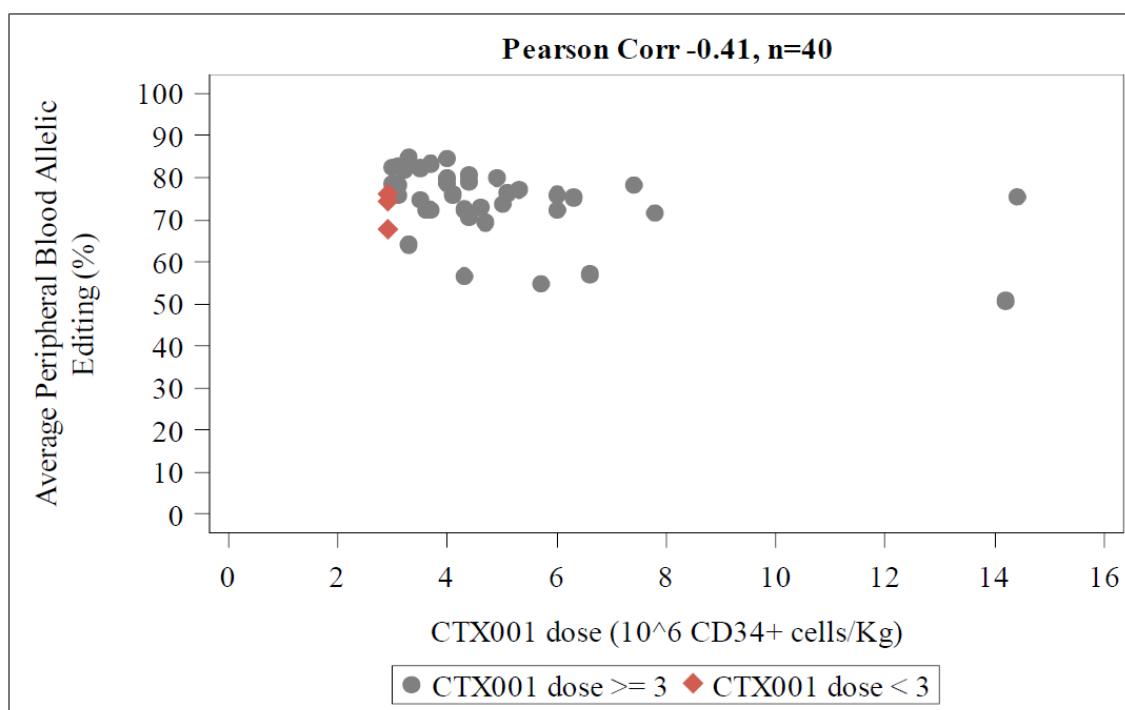


Note: The 3 subjects that received below the protocol specified dose (i.e.,  $< 3 \times 10^6$  CD34+ cells/kg) are identified by red diamond symbols. Source: Figure 1; Response to Clin Pharm IR#1

**Figure 2 Average Bone Marrow Allelic Editing (%) and Average Peripheral Blood Allelic Editing (%) Versus Exa-cel Dose ( $\times 10^6$  CD34+ cells/kg) (Studies 121 and 131, [SCD]FAS)**

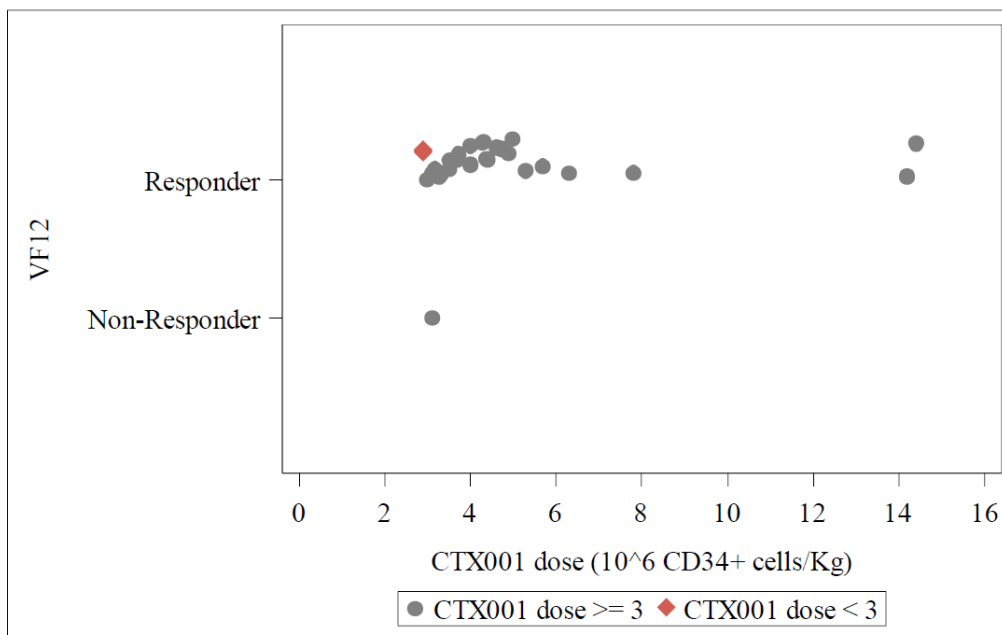


**b) Average editing in PB versus exa-cel dose**

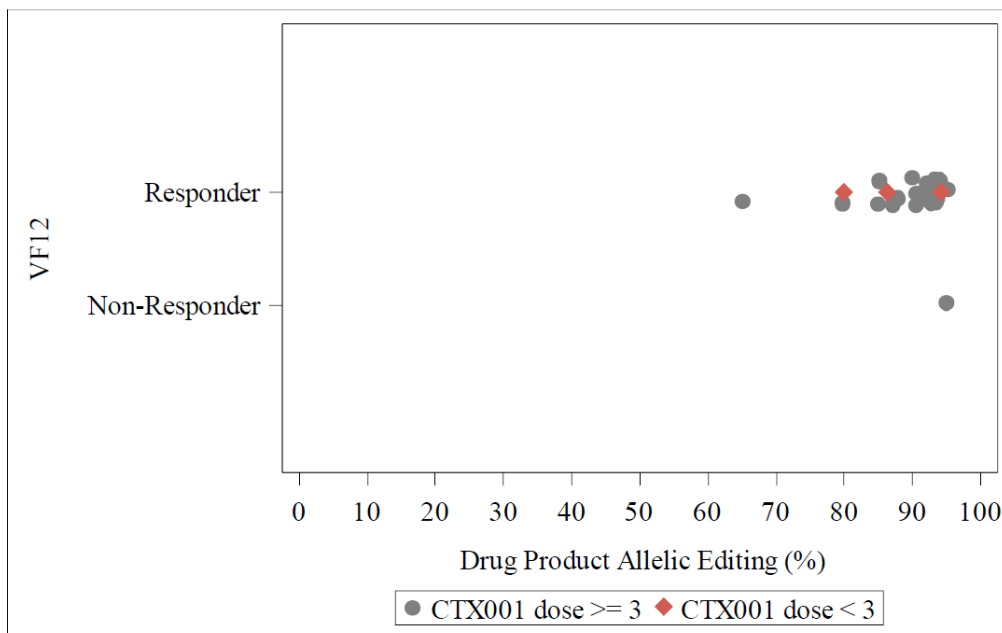


Source: Figure 2; Response to Clin Pharm IR#1

**Figure 9: VF12 Versus CASGEVY Dose ( $\times 10^6$  CD34+ cells/kg) and Allelic Editing (%) in Drug Product (Studies 121 and 131, [SCD]PES)**



**b) VF12 versus allelic editing in DP**



Source: Figure 4; Response to Clin Pharm IR#1

#### 6.4.3. HbF% versus VF12

Previous published studies suggest that individuals with SCD who co-inherit hereditary persistence of fetal hemoglobin (HPFH) with HbF levels  $\geq 20\%$  have little or no VOC. Based on this observation the relationship between HbF%  $\geq 20\%$  at Month 6 vs VF12 was evaluated (as Table 4). In the PES, 28 subjects had Month 6 HbF available and are all  $\geq 20\%$ ; no subject had HbF  $< 20\%$ . Two subjects with missing HbF at Month 6 (both subjects had HbF [%]  $> 20\%$  at Month 5) were not included in the analysis. Twenty-seven of 28 (96.4%) subjects in the PES with HbF (%)  $\geq 20\%$  at Month 6 achieved VF12 (Table 4).

The HbF (%) data were available for all subjects in Study 121 who had an assessment of HbF (%) at Month 6. Thirty-eight of 44 subjects in the FAS had Month 6 HbF (%) data available at the time of the data cutoff date. In addition to the 28 subjects in PES, there are 10 additional subjects with HbF at Month 6. Among these 10 subjects, one subject who died due to COVID-19 infection that resulted in respiratory failure was excluded from the analyses as the Month 6 HbF (%) data were diluted by frequent RBC transfusions while hospitalized, thus reducing the number to 9 subjects. Among the 9 subjects with Month 6 HbF (%) and not yet evaluable VF12, all had HbF (%) of  $\geq 20\%$ . Based on a 96% probability (as observed in the PES in Study 121 [29 of 30 subjects (96.7%) achieved VF12]) of these subjects who achieved HbF (%) of  $\geq 20\%$ , it is predicted that 8 of these 9 subjects will achieve VF12. By including the subjects who have already achieved VF12 (N = 29) with those who are predicted to achieve VF12 using HbF (%) at Month 6 (n = 8), it is expected that 37 of the 39 (94.9%) subjects will achieve VF12, consistent with what was already reported for the primary efficacy endpoint for subjects in the PES (96.7%).

**Reviewer comment: Prediction of VF12 based on Month 6 HbF%  $\geq 20\%$  allowed to include 9 additional subjects for efficacy analysis. The predicted efficacy of 95% (37 of 39 subjects) is consistent with the observed  $> 95\%$  efficacy. However, data are limited for robust correlative evaluation of HbF% vs VF12. For a valid correlative analysis it is important to include data with HbF%  $< 20\%$  and understand if there is**

a relationship with VF12. Also, it remains unknown why one individual with HbF% >20% not responding.

**Table 4: Summary of HbF (%) and VF12 Response (Study 121 PES)**

HbF (%) at Month 6	VF12	
	Responder	Non-responder
≥20 %	27	1
<20 %	0	0

Source: Table 3; Response to Clin Pharm IR#1

#### 6.4.4. Population Modeling of HbF%

Population pharmacodynamic (popPD) analysis of HbF% was conducted to characterize longitudinal change in HbF% and predict HbF% at Months 24 and 48 following treatments with CASGEVY. The population longitudinal nonlinear mixed-effects (NLME) model was built using the [FAS dataset (N= 42 subjects with data out to Month 42 across Studies 121 and 131). The initial structural model is based on empirical sigmoidal function (Emax) which was further refitted with offset function following visual observation of small decline of HbF after reaching peak level around 6 months (see the appendix for details).

The popPD model was used to quantify HbF% at Months 24 and extrapolate to Months 48. Based on simulations, the median predicted Month 24 HbF% was 42.1% (range: 25.7% to 49.9%), with 97.5% of the subjects at or above 32.1%. The popPD model was also used to screen for potential impact of intrinsic factors (age, race, sex, weight), extrinsic factors (busulfan cAUC), and manufacturing attributes (administered CD34+ cells/kg, percent allelic editing in drug product) against the Empirical Bayes Estimates (EBEs) of the time to half-maximal HbF (%) and steady state HbF (%) parameters from the reported base popPD model. Updated data (June 14, 2023, data cutoff) were used for covariate screening. Across all the evaluations of intrinsic, extrinsic, and manufacturing factors, none of the categorical comparisons reach significance at the  $P = 0.05$  level. Overall, for the range of factors explored no clinically relevant effects of intrinsic, extrinsic, or manufacturing factors are observed.



Reviewer comments: The empirical popPD model reasonably described the observed HbF% vs time profile. The model predicted HbF% at Month 24 was consistent with the observed data. Also, the popPD finding on the lack of correlation between manufacturing attributes and intrinsic factors versus HbF% is consistent with the result of traditional correlative assessment. However, the current popPD analysis cannot be used for extrapolation of HbF% up to Months 48 due to limited data with long-term follow-up and lack of mechanistic component to describe the variability of HbF%.

### **Overall Summary:**

The following are key summary of the clinical pharmacology findings:

- The mean proportion of alleles with the intended genetic modification in peripheral blood was generally maintained  $\geq 70\%$  from Month 2 onward, through the duration of follow-up in Studies 121 and 131.
- The mean (SD) proportion of total Hb comprised of HbF (%) was 36.9% (9.0%) at Month 3 and was maintained at  $\geq 40\%$  from Month 6 over the duration of follow-up.
- Correlative analysis demonstrates a correlation of the earlier timepoint (Month 6) with later timepoints (e.g., Month 12 & 24) for parameters such as HbF% and allelic editing in bone marrow and peripheral blood.
- No relevant dose-response relationship was identified for HbF% and clinical efficacy (VF12).
- The empirical population pharmacodynamic model reasonably described the observed HbF% vs time profile up to Month 24.

Overall, dose-response and correlative assessment did not identify CASGEVY dose as a factor affecting HbF% or clinical efficacy (VF12) based on the limited clinical data. The product allelic editing and % net increase in gamma globin expression appears to correlate with in vivo persistence of gene edited cells. However, the available data don't allow to derive a threshold of in vivo persistence that correlates with HbF (%) or VF12.

## 7. Appendix

### 7.1. Study#1: CTX001-121

**Study status:** Ongoing (Interim analysis data cutoff date: February 10, 2023 & updated June 14, 2023)

**Title:** A Phase 1/2/3 Study to Evaluate the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified CD34+ Human Hematopoietic Stem and Progenitor Cells (CTX001) in Subjects With Severe Sickle Cell Disease (#CTX001-121)

**Objectives:** The primary objective of the study is to evaluate the safety and efficacy of a single dose of autologous CRISPR/Cas9 modified CD34+ human hematopoietic stem and progenitor cells (hHSPCs) (exagamglogene autotemcel [CASGEVY], formerly CTX001) in subjects with severe sickle cell disease (SCD). The primary efficacy endpoint was the proportion of subjects who had not experienced any severe VOC for at least 12 consecutive months (VF12) after CASGEVY infusion. The evaluation of VF12 started 60 days after the last RBC transfusion for post-transplant support or SCD management.

The secondary objective of the study is to assess the effects of infusion of CASGEVY on disease-specific events and clinical status and quantify gene editing efficiency.

The exploratory objective is to assess the ability of biomarkers to characterize CASGEVY effect and predict treatment outcomes.

**Methodology:** 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 severe SCD. The study evaluated the safety and efficacy of a single dose of CASGEVY. For each subject, the study was conducted in 4 stages (See schematic below):

- Stage 1 included Screening and the pre-mobilization period,
- Stage 2 included mobilization, autologous CD34+ stem cell collection, and CASGEVY manufacture and disposition,
- Stage 3 included myeloablative conditioning and CASGEVY infusion, and

- Stage 4 included post-infusion in-hospital follow-up (until neutrophil engraftment, Stage 4A) and post-discharge follow-up (approximately 2 years, Stage 4B).

All subjects who received CASGEVY were asked to enroll into the long-term follow-up study (#Study 131) after completion or withdrawal/discontinuation from Study 121.

#### **Study Disposition & Demographics:**

- A total of 63 subjects were enrolled. At the time of the data cutoff date, 58 subjects had started mobilization and were included in the Safety Analysis Set (SAS) and 42 subjects had received CASGEVY infusion and were included in the full analysis set (FAS).
- One subject discontinued the study after CASGEVY infusion because of death due to respiratory failure after COVID-19 infection.
- Fifteen subjects discontinued the study before CASGEVY infusion: 5 subjects before mobilization and 10 subjects after the start of the mobilization but before start of conditioning.
- Twenty subjects were evaluable for the primary efficacy set (PES). PES included all subjects were followed for at least 16 months after CASGEVY infusion and for at least 14 months after completion of the RBC transfusions for post-transplant support or SCD management.
- For the 42 subjects in the FAS, the median (range) age was 20 (12 to 34) years of age, with 12 (28.6%) subjects  $\geq 12$  and  $< 18$  years of age.
- Most subjects were Black or African American (85.7%). Subjects were approximately balanced by sex. Of the 42 subjects in the FAS, 38 (90.5%) subjects had  $\beta^S/\beta^S$  genotype, 3 (7.1%) subjects had  $\beta^S/\beta^0$  genotype, and 1 (2.4%) subject had  $\beta^S/\beta^+$  genotype.

#### **Study Treatments:**

- Subjects received plerixafor at a dose of 0.24 mg/kg via subcutaneous injection 2 to 3 hours before the start of apheresis.
- Busulfan was administered IV through a central venous catheter daily at a starting dose of 3.2 mg/kg/day for 4 consecutive days (based on body weight collected within 7 days before the first day of busulfan administration). Once daily (qd) dosing was the preferred schedule, but the busulfan dose regimen could be adjusted to be given every 6 hours

<p>(q6h) per site's standard practice. For subjects &lt;34 kg, weight-based starting dose recommendations for busulfan were: (i) 1.1 mg/kg q6h or 4.4 mg/kg qd (for subjects weighing 16 to 23 kg), and (ii) 0.95 mg/kg q6h or 3.8 mg/kg qd (for subjects weighing &gt;23 to 34 kg)</p> <ul style="list-style-type: none"> <li>• CASGEVY Infusion: The minimum dose of exac-cel was <math>3 \times 10^6</math> CD34+ cells/kg based clinical experience from autologous transplantation. The maximum cell dose of <math>20 \times 10^6</math> CD34+ cells/kg was selected based on manufacturing capabilities. Subjects received the entire dose of CASGEVY on Day 1 via infusion through a central venous catheter.</li> <li>• The single dose of CASGEVY was given at least 48 hours and within 7 days after the last busulfan dose</li> </ul>
<p><b>Pharmacokinetic/Pharmacodynamic Assessment for Busulfan:</b> Blood samples were collected on Days 1 and 3 of conditioning to determine plasma concentrations of busulfan. The dose of busulfan could be adjusted based on the pharmacokinetics (PK) of the first busulfan dose to maintain appropriate levels (e.g., achieve target cAUC) for myeloablation. In the context of myeloablative conditioning and hematopoietic recovery, neutrophil and platelet engraftment was each considered assessments of pharmacodynamics (PD) and were evaluated for PK/PD relationships with busulfan.</p>
<p><b>Pharmacokinetic/Pharmacodynamic Assessment for CASGEVY:</b> Blood samples were collected for evaluation of PK/PD (using central laboratories) at pre-infusion (before mobilization) and after ex-cell infusion as follows:</p> <ol style="list-style-type: none"> <li>1. Proportion of alleles with intended genetic modifications in peripheral blood: <ul style="list-style-type: none"> <li>• Month 1, 2, 3, 4, 5, 6,9 ,12, 18, and 24.</li> </ul> </li> <li>2. Hb (absolute value), HbF (absolute value and %) concentrations and F-cells: <ul style="list-style-type: none"> <li>• Month 1, 2, 3,4, 5, 6,9 ,12, 15,18, 21 and 24.</li> </ul> </li> <li>3. Exploratory biomarkers: <ul style="list-style-type: none"> <li>• Month 3, 6,12,18 and 24</li> </ul> </li> </ol>

## 7.2. Study#2- CTX001-131

**Study Status:** Ongoing (Interim analysis data cutoff date: February 10, 2023 & updated on June 14, 2023)

<b>Title:</b> A Long-term Follow-up Study of Subjects With $\beta$ -thalassemia or Sickle Cell Disease Treated with Autologous CRISPR-Cas9 Modified Hematopoietic Stem Cells (CTX001)
<b>Objectives:</b> The primary objective is to evaluate long-term safety up to 15 years after exagamglogene autotemcel (CASGEVY; formerly CTX001) infusion, in subjects who received CASGEVY  The secondary objective is to evaluate efficacy of CASGEVY up to 15 years after CASGEVY infusion, in subjects who received CASGEVY for treatment of transfusion-dependent $\beta$ -thalassemia (TDT) or severe sickle cell disease (SCD)
<b>Methodology:</b> This is an ongoing, multi-site, open-label, rollover study designed to evaluate the long-term safety and efficacy of CASGEVY in subjects who received CASGEVY in a parent study for a total follow-up of 15 years after CASGEVY infusion. Studies 111 and 121 are the first-in-human Phase 1/2/3 studies with CASGEVY in subjects 12 to 35 years of age. In each of the two parent studies, subjects are followed for up to approximately 2 years (to Month 24) after CASGEVY infusion. All subjects who received CASGEVY infusion who completed or discontinued from a parent study were asked to participate in Study 131. This study report focus subjects enrolled in study 121 and followed over 2 years as part of Study 131.
<b>Study Disposition:</b>  As of the current data cutoff dates, 8 subjects with SCD from Study 121 have enrolled in Study 131.
<b>Pharmacokinetic/Pharmacodynamic Analysis:</b> <ol style="list-style-type: none"><li>1. Hemoglobin (Hb; absolute value) and fetal hemoglobin (HbF; in absolute value and %) concentrations:</li><li>2. Proportion of alleles with intended genetic modifications present in peripheral blood</li><li>3. Exploratory biomarkers: Hb fractionation (supportive of Hb and HbF assessments), circulating erythrocytes expressing <math>\gamma</math>-globin (HbF; F-cells), and blood erythropoietin (Epo) level.</li></ol>



### 7.3. Study #3- Population Pharmacodynamic Modeling of HbF (%)

**Objective:** to develop a population pharmacodynamic (popPD) non-linear mixed effects (NLME) model of the time course of HbF% in SCD subjects after CASGEVY administration, and use it to:

- Characterize the longitudinal changes in HbF%
- Predict HbF% levels at 24 months and up to 48 months

**Data:** The modeling dataset included 40 SCD subjects, ages 12-34, who received CASGEVY in Study 121 and Study 131. A total of 350 observations (HbF%) over the follow-up duration of 2 to 41 months. The dataset used in this base model analysis was derived from the SCD full analysis set (FAS). There were no HbF% data below the limit of quantification (BLQ) in the analysis dataset.

#### **Structural Model:**

Base model development to describe the pharmacology of CASGEVY consisted of population longitudinal NLME repeated-measures models to predict HbF% levels in SCD subjects from studies 121 and 131. Per the analysis plan, modeling began with use of a sigmoidal *E<sub>max</sub>* model as the fixed effects structure, as in Equation (1) below:

$$HbF\% = HbF\%_{T0} + \frac{(HbF\%_{max} - HbF\%_{T0}) \times Time^{\gamma}}{Time^{\gamma} + T_{50}^{\gamma}} \quad (1)$$

where:

- dependent variable *HbF%* is fetal hemoglobin (% of total hemoglobin)
- *Time* is the duration (in days) since the first observation after CASGEVY dosing
- *HbF%<sub>T0</sub>* is *HbF%* at the first observation after CASGEVY dosing
- *HbF%<sub>max</sub>* is the maximum *HbF%*
- *T<sub>50</sub>* is the duration (in days) needed to reach 50% of *HbF%<sub>max</sub>*

- $\gamma$  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 378 (range: 132 to 978) days and that HbF% was highly variable. These observations, together with pre-treatment regimens required prior to CASGEVY dosing (cellular mobilizations and myeloablation, resulted in the baseline HbF% measurements not being considered reliable representations of HbF% levels at, or immediately following, CASGEVY administration. Therefore, the earliest post-dose HbF% observation timepoint (28 days) was selected to represent time-zero for modeling and used to estimate  $HbF\%T_0$ . While HbF% levels at this timepoint are affected by the prior administration of CASGEVY, it more closely represents the baseline condition relative to dose administration. Additionally, as subjects' cells are being genetically modified by the CRISPR-Cas9 therapy, it is a reasonable assumption that the choice of baseline exerts minimal influence on the eventual course of HbF% over time.

**Reviewer comment: The baseline definition for the popPD analysis deviate from the protocol definition of baseline for HbF% (i.e., the study 121 protocol define baseline as the most recent non-missing measurement collected before start of mobilization). To address this issue the applicant conducted sensitivity analysis by comparing model performance with and without the protocol-defined baseline HbF (%) in the dataset. The sensitivity analysis showed negligible difference in model predicted value of HbF% suggesting that the applicant assumption of baseline is reasonable.**

### **Model Diagnostics:**

Base model goodness-of-fit was assessed by graphical examination and visual predictive checks (VPCs). For VPC, simulations were performed using the base model, fixed and random effects, and the same dataset structure. The original data set was used as a template for simulation input. Simulations were performed to generate 1000 simulated observations at each timepoint. A graphical comparison was then made between the



observed data and the model predicted median, 2.5<sup>th</sup>, and 97.5<sup>th</sup> prediction interval over time.

### **Model-based Simulations:**

The base model was used to predict in-cohort individual and population typical HbF% levels following CASGEVY administration for each subject included in the analysis and extrapolated up to Month 48.

### **Results and Discussion of popPD Modeling:**

Of the 42 subjects in the FAS, one subject did not have any post-dose collections of HbF% in the dataset and was excluded. For the 41 remaining subjects, additional evaluations were made to account for transfusion histories, as transfusions impact HbF% levels. Subjects who received more than 3 units of red blood cells during a transfusion visit had subsequent samples within a 60-day window was excluded. One subject had an exchange transfusion on Day 117, requiring seven units of red blood cells and thus the three subsequent timepoints within 60 days of Day 117 were excluded from the analysis. Another subject, who died due to respiratory failure resulting from COVID-19 infection, received repetitive transfusions starting at Day 115 and was excluded from analysis. Lastly, all CASGEVY pre-dose data were excluded. In total 40 subjects with 350 HbF% observations were included for the popPD analysis.

The initial growth function model (equation 1) overpredicted HbF% values beyond 6 months. To accommodate the decline phase after peak level an additional fixed effects structural components describing offset function from the maximum HbF% was included (equation 2).

$$HbF\%_{off} = 1 - \frac{O_{max} \times Time^{\nu}}{Time^{\nu} + OT_{50}^{\nu}} \quad (2)$$

where:

- $HbF\%_{off}$  is the fractional offset from maximum HbF% over time
- $O_{max}$  is the maximum fractional offset from maximum HbF%

- $OT50$  is the duration (in days) needed to reach 50% of  $O_{max}$
- $\nu$  is the Hill coefficient reflecting the sigmoidicity around  $OT50$

The structural model parameters and inter-individual variances for the final base model (i.e., sigmoidal with offset) are summarized in Table 5. The diagnostic plots and visual predictive checks (VPC) for the selected base model (sigmoidal with offset function) are displayed in Figure 10&11. The VPC demonstrate that the model appropriately captures the observed data at all timepoints, including beyond 6 months (Figure 12), with the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentile of the VPC being more consistent with the observed data.

The popPD model was used to screen for potential impact of intrinsic factors (age, race, sex, weight), extrinsic factors (busulfan cAUC), and manufacturing attributes (administered CD34+ cells/kg, percent allelic editing in drug product) against the Empirical Bayes Estimates (EBEs) of the time to half-maximal HbF (%) and steady state HbF (%) parameters from the reported base PopPD model. Data utilized are from the 14 June 2023 data cutoff. Across all the evaluations of intrinsic, extrinsic, and manufacturing factors, none of the categorical comparisons reach significance at the  $P = 0.05$  level. Across the range of factors explored no clinically relevant effects of intrinsic, extrinsic, or manufacturing factors are observed and thus, none were formally entered in the pop PD model as predictors of HbF%.

Finally, the base model was used to forecast the in-sample expectation of HbF% at Months 24 and extrapolate to Months 48 (Figures 13). Based on simulations, the median predicted Month 24 HbF% was 42.1% (range: 25.7% to 49.9%), with 97.5% of the subjects at or above 32.1%. Similarly, at Month 48, these 39 patients are predicted to have HbF% sustained above  $\geq 31.9\%$ , with the full analyzed cohort median (range) being 42.0% (25.5% to 50.0%) HbF%, demonstrating the stability of HbF% levels over time.

Reviewer comments: The objective function value (OFV) for the base sigmoidal model (1270) was slightly higher than the sigmoidal model with offset (1113) and model diagnostic plots were improved by including the offset function. However, the sigmoidal model with offset function has high relative standard error (%RSE) for  $HbF\%_{max0}$  (53.1%) and %RSE for  $O_{max}$  (88.1%). It is also not clear why the initial declining HbF% in some subjects require a separate three parameters sigmoidal offset function. The in-

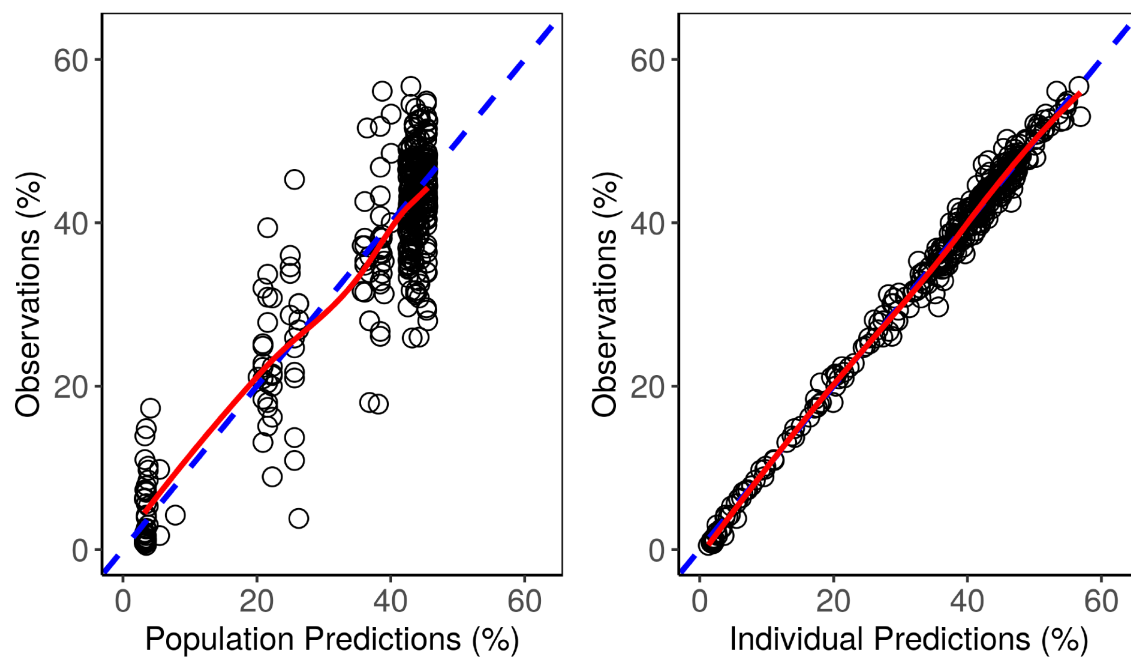
sample simulation up to 24 Months appear to be acceptable as this is informed by available clinical data. Also, the popPD finding on the lack of correlation between manufacturing attributes and intrinsic/extrinsic factors versus HbF% is consistent with the result of traditional correlative assessment. Overall, the current model is viewed as exploratory and not appropriate for extrapolating HbF% to Month 48 due to limited data, high inter-individual variability, and lack of mechanistic quantitative information. We recommend to further explore the base model by including biologically plausible structural model or covariates to better quantify the inter-individual variability in HbF%.

**Table 5: Summary of Sigmoidal Model with Offset Function**

		Estimate	RSE%	[95% CI]	Shrinkage <sub>SD</sub> %
<b>Structural model parameters</b>					
$\theta_{HbF\%_{max0}}$	Typical value of scaled logit maximum HbF%	0.839 <sup>†</sup>	53.1	[-0.0336, 1.71]	-
$\theta_{HbF\%_{T0}}$	Typical value of scaled logit time-zero HbF%	-3.42 <sup>†</sup>	6.07	[-3.82, -3.01]	-
$\theta_{T_{50}}$	Typical value of time (days) to half maximum HbF%	52.7	10.9	[41.5, 64]	-
$\theta_{\gamma}$	Typical value of sigmoidicity	1.65	7.02	[1.42, 1.88]	-
$\theta_{O_{max}}$	Typical value of logit-transformed offset maximum	-0.441 <sup>†</sup>	88.1	[-1.2, 0.32]	-
$\theta_{OT_{50}}$	Typical value of time (days) to half offset maximum	120	19.6	[73.9, 166]	-
$\theta_{\nu}$	Typical value of offset sigmoidicity	2.09	25.8	[1.03, 3.15]	-
<b>Inter-individual variances</b>					
$\Omega_{HbF\%_{max0}}$	Variance of scaled logit maximum HbF%	0.174 <sup>†</sup>	77.6	[-0.0905, 0.438]	10.0
$\Omega_{HbF\%_{T0}}$	Variance of scaled logit time-zero HbF%	0.84 <sup>†</sup>	23.7	[0.451, 1.23]	7.0
$\Omega_{T_{50}}$	Variance of time to half maximum HbF%	0.0549 <sup>‡</sup>	35.9	[0.0163, 0.0935]	20.5
$\Omega_{\gamma}$	Variance of sigmoidicity	0.0987 <sup>‡</sup>	33.3	[0.0343, 0.163]	11.4
<b>Residual error variance</b>					
$\Sigma$	Variance of additive residual error	2.83	14.7	[2.02, 3.65]	19.3

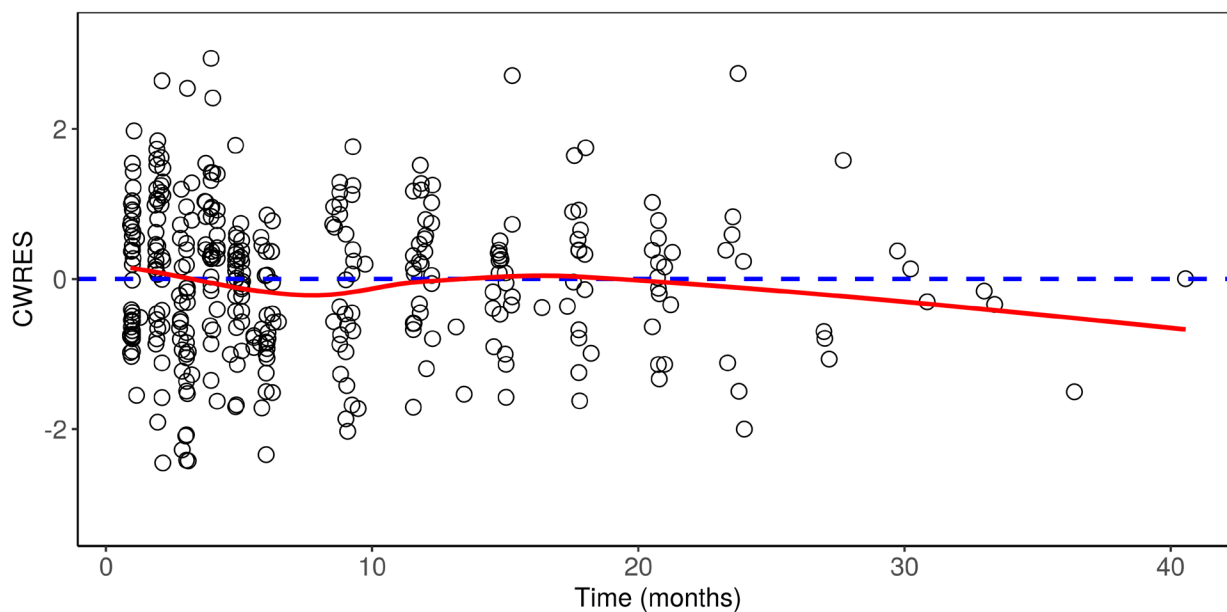
Source: Table 7.3; Report T068-SCD

**Figure 10: HbF% observations vs. predictions (sigmoidal model with offset function)**



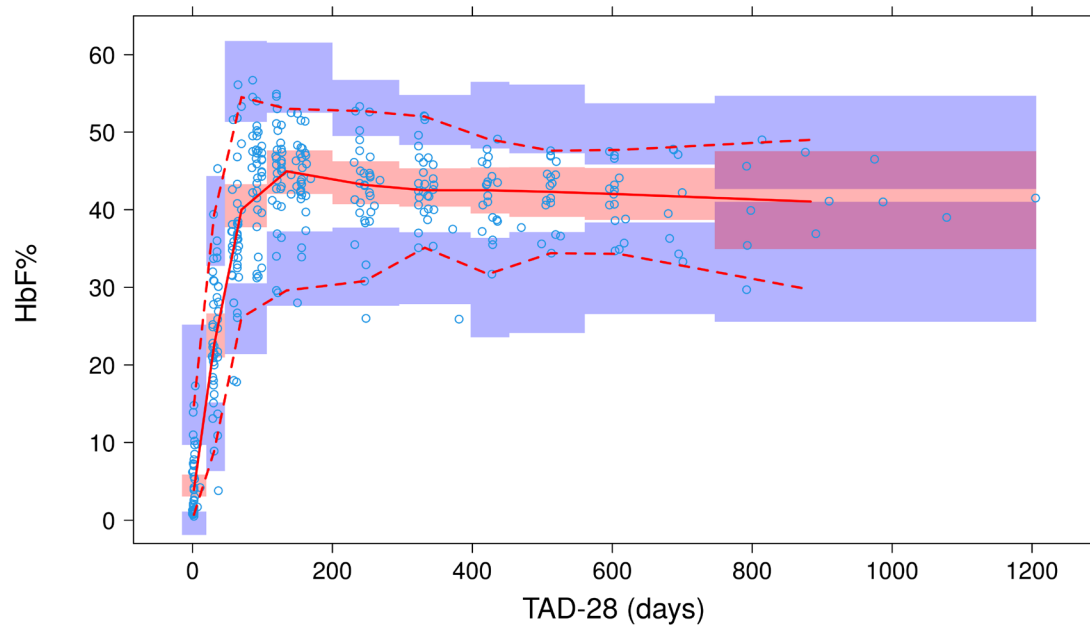
Source: Figure 7.3; Report T068-SCD

**Figure 11: Individual and conditional weighted residuals of predictions over time**



Source: Figure 7.5; Report T068-SCD

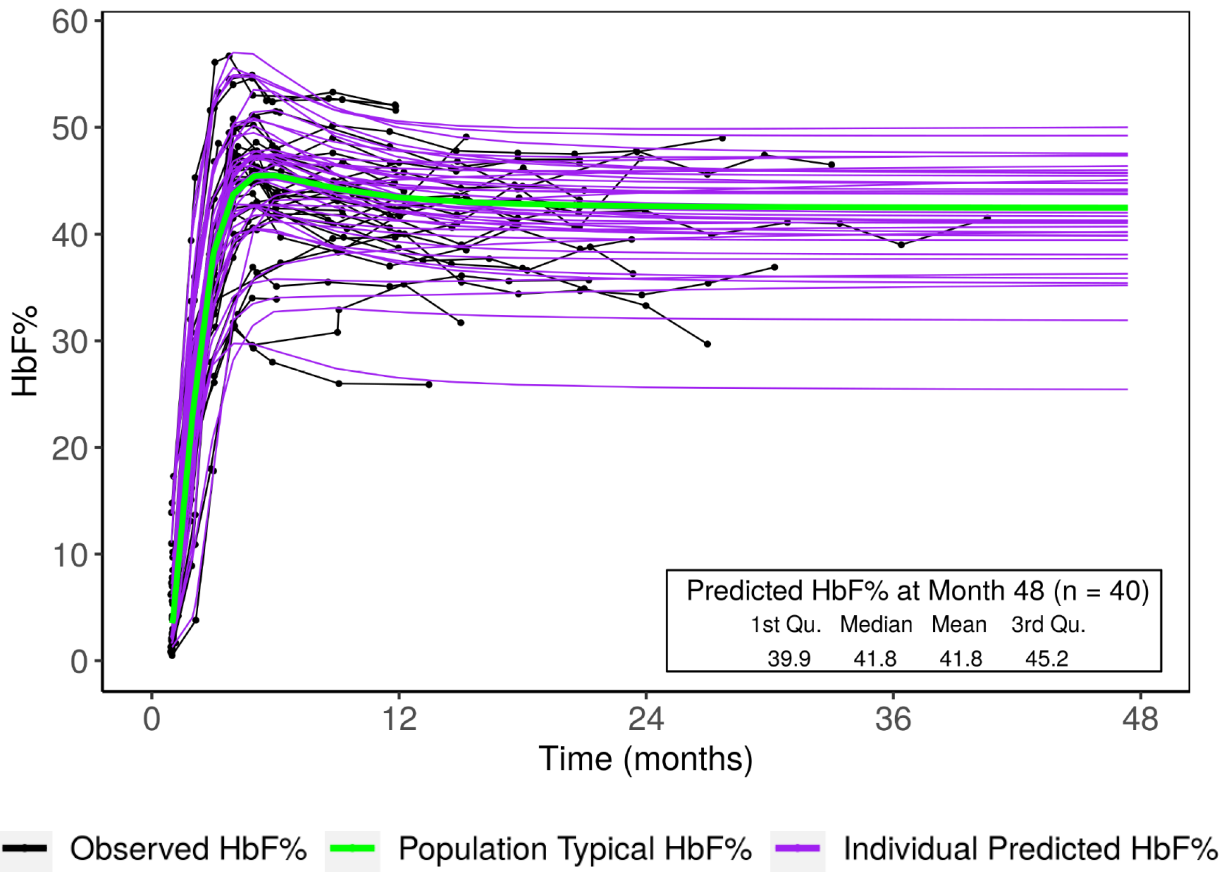
**Figure 12: Visual predictive check of model including offset function**



Note: The median and the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles of the observed data (blue dots) are indicated with the solid red curve and the dashed red curves, respectively. 95% confidence intervals of predictions are displayed as areas in rose (median) and periwinkle (2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles). 1000 simulated replicates were used for VPC.

Source: Figure 7.6; Report T068-SCD

**Figure 13: Individual and population typical HbF% predictions to month 48**



Source: Figure 7.8; Report T068-SCD