BLA Clinical Review Memorandum

Application Type	Original BLA
STN	125787/0
CBER Received Date	April 3, 2023
PDUFA Goal Date	December 8, 2023
Division / Office	OTP CBER
Priority Review (Yes/No)	Yes
Reviewer Name(s)	Karl Kasamon MD
Review Completion Date / Stamped Date	12/08/2023
Supervisory Concurrence	Prasad Mathew MD Nicole Verdun MD
Applicant	Vertex Pharmaceuticals Inc.
Established Name	Exagamglogene autotemcel
(Proposed) Trade Name	CASGEVY
Pharmacologic Class	Autologous Hematopoietic Stem and progenitor Cells (HSPC)
Formulation(s), including Adjuvants, etc.	Autologous CD34+ Hematopoietic Stem Cells edited ex vivo with CRISPR/Cas9
Dosage Form(s) and Route(s) of Administration	Exagamglogene autotemcel is a cell suspension for infusion. Single cell dose of ≥3.0 × 10^6 CD34+ cells/kg to be provided via intravenous (IV) infusion
Dosing Regimen	Single treatment
Indication(s) and Intended Population(s)	Treatment of sickle cell disease (SCD) in patients 12 years and older with recurrent vaso-occlusive crises (VOCs)
Orphan Designated (Yes/No)	Yes

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GLOSSARY

AC	advisory committee
ACS	acute chest syndrome
AE	adverse event
allo-HSCT	allogeneic hematopoietic stem cell transplantation
AUC	area under the concentration-time curve
BIMO	Bioresearch Monitoring
BLA	biologics license application
BCL11A	B-cell lymphoma/leukemia 11A (transcription factor)
CBER	Center for Biologics Evaluation and Research
CMC	chemistry, manufacturing, and controls
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats associated 9 nucleases
EAC	Endpoint Adjudication Committee
ECMO	extra corporeal membrane oxygenation
Exa-cel	exagamglogene autotemcel
FAS	full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
G-CSF	granulocyte-colony stimulating factor
gRNA	guide ribonucleic acid
GVHD	graft-versus-host disease
HbA	adult hemoglobin
Hb	hemoglobin
HbF	fetal hemoglobin
HbS	sickle hemoglobin
HF12	no hospitalization for sVOCs sustained ≥12 months post exa-cel
HPFH	hereditary persistence of fetal hemoglobin
HSC	hematopoietic stem cell
HSPC	hematopoietic stem and progenitor cell
HSCT	hematopoietic stem cell transplantation
IA	interim analysis
IV	intravenous
IR	information request
LDH	lactate dehydrogenase
N	total sample size/number of subjects
n	size of subsample
NE	neutrophil engraftment
NSAID	non-steroidal anti-inflammatory drug
NSG	NOD/SCID/IL2Rgnull
PE	platelet engraftment
PES	primary efficacy set
PMR	postmarketing requirement
PREA	Pediatric Research Equity Act

PRO	patient-report outcome
Q6h	every 6-hour
QOL	quality of life
RBC	red blood cell
RNP	ribonucleoprotein
SAE	serious adverse event
SCD	sickle cell disease
SD	standard deviation
sVOC	severe VOC
TDT	transfusion-dependent β-thalassemia
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
USPI	United States Prescribing Information
VF12	absence of sVOCs ≥12 consecutive months after exa-cel infusion
VOC	vaso occlusive crisis
VOLD	veno occlusive liver disease

1. EXECUTIVE SUMMARY

Vertex Pharmaceuticals Inc. has submitted Biologics License Application (BLA) 125787, for the licensure of exagamglogene autotemcel (referred to as exa-cel with trade name CASGEVY) for the treatment of sickle cell disease (SCD) in patients 12 years and older with recurrent vaso-occlusive crises (VOCs). Exa-cel is composed of autologous CD34+ hematopoietic stem and progenitor cells (HSPCs) modified ex vivo with clustered regularly interspaced short palindromic repeats associated 9 nucleases (CRISPR/CAS9) suspended in cryopreservative solution. The HSPCs in exa-cel are edited to engraft and mature into erythroid lineage cells with reduced B-cell lymphoma/leukemia 11A (BCL11A) expression. Reduced BCL11A expression results in an increase in γ globin expression and fetal hemoglobin (HbF) production in red blood cells (RBCs). Increased HbF expression reduces intracellular sickle hemoglobin (HbS) concentration in patients with SCD, preventing RBCs from sickling and thus reversing the underlying cause of disease, thereby reducing or eliminating VOCs. The recommended regimen is a single exa-cel dose of $\geq 3x10^6$ CD34+ cells/kg administered intravenously (IV) after full myeloablative conditioning with busulfan.

SCD is a group of hemoglobinopathies that includes sickle cell anemia, sickle beta-plus thalassemia, sickle beta-zero thalassemia, and sickle hemoglobin C disease.[1] SCD affects around 80,000 individuals in the U.S. (Centers for Disease Control and Prevention 2023), and the most severe form, caused by a homozygous mutation (HbSS, βS/βS), accounts for two-thirds of U.S. cases. A single nucleotide substitution in the β-globin chain leads to polymerization of HbS molecules when de-oxygenated, impacting the structure and function of RBCs. The sickled RBCs are inflexible and adhesive, forming heterocellular aggregates that lead to tissue ischemia and chronic organ damage referred to as vaso-occlusion. VOCs are severe pain events recurrently experienced by patients with SCD due to vaso-occlusion. Other hallmark manifestations due to vaso-occlusion include acute chest syndrome (ACS), priapism, hepatic and splenic sequestration, and hemolytic anemia. SCD leads to progressive organ damage including strokes and pulmonary, cardiac, and renal diseases, and ultimately shortens the survival of patients with SCD by approximately two decades compared with unaffected peers (Williams and Thein 2018; Johnson et al. 2023).

Evidence to support effectiveness and safety of exa-cel comes from one uncontrolled study (Study 121), and the roll over, long-term safety follow-up study, Study 131. Study 121 is an ongoing Phase 1/2/3 study in which 44 subjects were given a single dose of exa-cel, after prerequisite mobilization and apheresis of HSPCs and myeloablation with busulfan. Eligible subjects had the βS/βS, βS/β0, or βS/β+ genotype and recurrent severe VOCs (sVOCs) and were observed for 24 months from exa-cel infusion before being able to enroll into Study 131 for up to 15 years of observation. The primary efficacy endpoint was

1 Sickle hemoglobin C will not be further addressed in this memo, as it was not studied in the studies included in this BLA

VF12 (absence of sVOCs for ≥12 months on study after exa-cel infusion, and ≥60-day washout from any RBC transfusions for SCD or autologous transplant management). Of 31 efficacy analysis eligible subjects, 29 achieved the primary efficacy endpoint, and 28 of these remained free of sVOCs for a mean duration of 22.3 (standard deviation [SD] 7.2) months. All 30 (100%) evaluable subjects achieved the key secondary efficacy endpoint of Study 121, remaining free from hospitalization for sVOCs for ≥12 consecutive months post exa-cel (HF12). The clinical efficacy endpoints were supported by pharmacodynamic endpoints demonstrating that the mean allelic editing in CD34+ cells of the bone marrow remained ≥80% from Month 6 through Month 24. Similarly, mean allelic editing was stable, generally maintaining ≥70% in peripheral blood from Month 2 through the duration of follow-up.

The safety profile observed was largely as expected with autologous hematopoietic stem cell transplant (HSCT) utilizing busulfan myeloablation, although engraftment of platelets was delayed. There was one death, unrelated to exa-cel. The reviewed safety data do not warrant Risk Evaluation and Mitigation Strategies. However, in addition to product labeling and routine pharmacovigilance, clinical safety postmarketing requirement (PMR) studies are being required to assess the long-term risk of hematologic malignancies related to insertional oncogenesis, as well as to further assess off-target editing by CRISPR/Cas9 gene therapy.

The BLA provides substantial evidence of safety and effectiveness for exa-cel for treatment of SCD in patients 12 years and older with recurrent VOCs, based on adequate and well-controlled trial data. The overall benefit-risk profile appears favorable and favors regular approval for the sought indication.

1.1 Demographic Information: Subgroup Demographics and Analysis Summary

Of the 63 subjects screened, a total of 44 had been infused with exa-cel and were included in the full analysis set (FAS), as of the time of the 90-day safety update with a data lock date of June 14, 2023. Their mean (range) age was 21.2 (12 to 34) years, with 12 adolescents (27.3%) <18 years of age. Most subjects (86.4%) were Black or African American; 54.5% were male. Thirty-six subjects (81.8%) were enrolled and treated at U.S. centers; the remaining 8 (18.1%) received treatment in Europe.

1.2 Patient Experience Data

Data Submitted in the Application

Check if Submitted	Type of Data	Section Where Discussed, if Applicable
☒	Patient-reported outcome	6.1.11.2
☒	Observer-reported outcome	
☐	Clinician-reported outcome	

☐	Performance outcome	
☐	Patient-focused drug development meeting summary	
☐	FDA Patient Listening Session	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☐	**If no patient experience data were submitted by Applicant, indicate here.**	
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting	
☐	FDA Patient Listening Session	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☒	Other: (please specify)	Advisory Committee

Quality of life (QOL) outcomes were further discussed in the secondary efficacy analysis at the bottom of Section 6.1.11.2.

Reviewer Comment:
The Applicant used a single arm study to evaluate efficacy of exa-cel. The patient-reported, QOL data were not evaluated as part of the application review for United States Prescribing Information *(USPI), given the limitations of QOL assessments in uncontrolled, open-label trials. As with time-to-event endpoints, interpretation of patient-reported outcomes is challenging in uncontrolled clinical trials, because it is unclear to what extent the outcomes can be ascribed to the treatment effect of studied regimen versus to underlying disease and patient characteristics.*

2. CLINICAL AND REGULATORY BACKGROUND

2.1 Disease or Health-Related Condition(s) Studied

SCD is a group of hemoglobinopathies which includes sickle cell anemia, sickle beta-plus thalassemia, sickle beta-zero thalassemia, and sickle hemoglobin C disease. Sickle hemoglobin C disease is not included in this review memorandum as sickle hemoglobin C disease was not studied in the clinical trials analyzed. SCD is marked by mutations of β-globin with production of HbS,

which differs from normal adult hemoglobin (HbA) due to substitution of valine for glutamic acid on the β-globin chain.

Clinical manifestations of the disease result from polymerization of deoxyhemoglobin in hypoxic conditions, leading to rigidity and sickling deformity of the RBCs within blood vessels. The SCD clinical course is characterized by hemolytic anemia, episodes of severe acute pain called VOCs, chronic pain, progressive pulmonary and renal failure, cardiovascular disease, strokes, and cognitive decline due to cerebrovascular disease.

In Africa, an estimated 290,000 children with sickle cell disease are born annually; 50 to 80% of these children do not survive to adulthood. As late as the 1970s, mortality of U.S. children diagnosed with SCD was poor, with approximately half dying before adulthood (Scott 1970). While the pediatric outcomes have improved, adults with SCD continue to experience substantially shorter survival compared with unaffected peers, with a median survival of 40 to 50 years, with few surviving into their 60s.

Although hydroxyurea, L-glutamine, voxelotor, and crizanlizumab, as well as chronic blood transfusions, can significantly improve the clinical course, their use is often inconsistent due to healthcare disparities. Even when used consistently, these therapies are not curative but only delay the progression of this disease. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can be curative but is only an option for ~20% of patients with SCD who have a matched donor.

2.2 Currently Available, Pharmacologically Unrelated Treatment(s)/Intervention(s) for the Proposed Indication(s)

The most common approaches to treatment of SCD remain use of opioids and other analgesics for management of acute and chronic pain, along with supportive treatments. Chronic RBC transfusions, hydroxyurea , and allo-HSCT have long track records of efficacy. More recently approved products also include crizanlizumab, voxelotor, and L-arginine. However, none of these are curative except for allo-HSCT, which has limited availability due to lack of donors.

2.3 Safety and Efficacy of Pharmacologically Related Products

No similar product is approved.

2.4 Previous Human Experience With the Product (Including Foreign Experience)

None.

2.5 Summary of Pre- and Post-Submission Regulatory Activity Related to the Submission

Regulatory history based on submissions from Applicant and communications with Applicant are shown in Table 1.

Table 1. Regulatory Background

Date	Regulatory Event
April 27, 2018	Original Submission
May 25, 2018	IND placed on Clinical Hold
October 10, 2018	IND removed from Clinical Hold
January 02, 2019	Exa-cel granted Fast Track designation
May 11, 2020	Exa-cel granted Orphan Drug designation
May 05, 2020	Exa-cel granted Regenerative Medicine Advanced Therapy designation
May 17, 2022	Type B meeting
April 03, 2023	BLA submitted
July 08, 2023	Additional efficacy data submitted at time of 90-Day Safety Update, with date of data lock June 14, 2023.

Abbreviations: BLA, biologics license application; exa-cel, exagamglogene autotemcel; IA2, interim analysis 2; IND, investigational new drug application; N, number of subjects in the specified group, or the total sample.

3. SUBMISSION QUALITY AND GOOD CLINICAL PRACTICES

3.1 Submission Quality and Completeness

The submission was adequately organized and integrated to accommodate the conduct of a complete clinical review without unreasonable difficulty. Inadequacies were resolved via use of information requests (IRs).

3.2 Compliance With Good Clinical Practices And Submission Integrity

The Applicant indicated that the trials were completed in multiple centers overseas and in the U.S. under IND 18143, in accordance with the regulations specified in 21 CFR 312, and were compliant with Good Clinical Practice (GCP), international ethical and scientific quality standards for the design, conduct, recording, and reporting of clinical trials involving human subjects (including Title 21, U.S. CFR Parts 50, 54, 56 and 312 Subpart D; the International Conference on Harmonisation (ICH) Guideline on GCP, E6; and the ethical principles outlined in the Declaration of Helsinki). The clinical trials included provisions for obtaining informed consent by all study subjects, and for ethical treatment of study subjects.

Bioresearch Monitoring (BIMO) inspections were issued for two domestic Clinical Investigator sites participating in the conduct of study 121. No significant problems impacting the data submitted were discovered. Please also see BIMO review memorandum.

3.3 Financial Disclosures

No significant financial interests or conflicts were identified that could potentially bias the conduct of the studies. A complete list of clinical investigators was provided.

Covered clinical study (name and/or number): Study 121, 131
Was a list of clinical investigators provided? ☐x Yes ☐ No (Request list from applicant)
Total number of investigators identified: 91 (including those on forms 3454 and 3455)
Number of investigators who are sponsor employees (including both full-time and part-time employees): 0
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 3
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 3 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator in sponsor of covered study: 0 Is an attachment provided with details of the disclosable financial interests/arrangements? ☐ Yes ☒ No (Request details from applicant) Is a description of the steps taken to minimize potential bias provided? ☒ Yes ☐ No (Request information from applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 0 Is an attachment provided with the reason? ☐ Yes ☒ No (Request explanation from applicant)

4. SIGNIFICANT EFFICACY/SAFETY ISSUES RELATED TO OTHER REVIEW DISCIPLINES

4.1 Chemistry, Manufacturing, and Controls

Please see the extensive CMC memo for additional information.

4.2 Assay Validation

Please see CMC review memorandum for details.

4.3 Nonclinical Pharmacology/Toxicology

The Applicant performed several preclinical evaluations of exa-cel, including in vitro pharmacology studies where CD34+ cells from healthy donors, edited using the SPY101- ribonucleoprotein (RNP), showed editing at the target genomic locus of the target BCL11A/GATA1 binding site, with genome editing frequencies

ranging from 60% to 92%. The Applicant also completed in vivo pharmacology evaluations, an in vivo pharmacokinetic study, and an in vivo toxicology and tumorigenicity study of CD34+ HSPCs obtained from healthy donors and edited with SPY101-RNP in irradiated NOD/SCID/IL2Rgnull (NSG) mice. Mice received single doses of 1x 10^6 cells/mouse and were followed for 20 weeks without significant adverse findings or tumor formation being noted. Reproductive toxicity studies, developmental studies, or carcinogenicity studies were not conducted with exa-cel, based on the characteristics and safety profile of the product. Please see the Pharmacology/Toxicology Review Memorandum for more details.

4.4 Clinical Pharmacology

4.4.1 Mechanism of Action

Starting around birth, the level of HbF expression steadily decreases over a couple of years, stabilizing in most humans at ≤2%. Hereditary persistence of fetal hemoglobin (HPFH) is characterized by uncommonly elevated HbF levels beyond childhood and is often marked by ameliorated or absent SCD complications in those with both HPFH and SCD. Exa-cel aims to recapitulate this outcome by reactivation of HbF expression to levels observed in individuals with SCD coinherited with HPFH (i.e., >20%).

According to the Applicant, it is expected that genetic editing by exa-cel will be persistent. The RNP complex composed of Cas9 and specific gRNA, SPY101, targets the binding site of transcription factor GATA1 in the non-coding erythroid lineage-specific enhancer region of the BCL11A gene on chromosome 2, where CRISPR endonuclease makes double-strand DNA breaks. Repair of these breaks by nonhomologous end joining produces insertions and deletions in DNA that disrupt GATA1 binding, decreasing BCL11A transcription in RBCs. By decreasing BCL11A, exa-cel causes an increase in γ-globin production and therefore a reduction in βS-globin. RBCs after exa-cel treatment are expected to contain approximately 30% to 50% HbF, which may potentially be therapeutic.

Reviewer Comments:
The targeting of BCL11A has theoretical concerns, given reports that BCL11A is a transcription factor playing important roles in a multitude of cellular functions, including maintaining hematopoietic stem cell (HSC) functions and regulating lymphoid development; its editing could lead to late complications (Yu et al. 2012; Luc et al. 2016). Additionally, BCL11A is considered a proto-oncogene (Weniger et al. 2006), and thus even careful disruption of the BCL11A gene may lead to dysregulation of BCL11A expression, resulting in tumorigenicity concerns (Yin et al. 2019). A large number of genes are potential targets of BCL11A in the chromatin immunoprecipitation sequencing (ChIP-seq) datasets from the ENCODE Transcription Factor Targets dataset; therefore, modulating BCL11A expression via genome editing may unintentionally impact the expression of these downstream target genes (Rouillard et al. 2016). Lastly, BCL11A may play a role in erythroid lineage development; therefore, BCL11a erythroid conditional

knockout mice are slightly anemic (Esteghamat et al. 2013) and BCL11A knockout in human CD34+ cells causes defective erythroid maturation (Chang et al. 2017). While purely theoretical, these risks need to be kept in mind while evaluating the safety of any CRISPR/Cas9 product with this therapeutic target.

4.4.2 Human Pharmacodynamics

The mean proportion of alleles with the intended genetic modification in peripheral blood was generally maintained ≥70% from Month 2 onward through the duration of follow-up in Studies 121 and 131. The mean (SD) proportion of total Hb composed of HbF (%) was 36.9% (9.0%) at Month 3 and was maintained at ≥40% from Month 6 over the duration of follow-up. Correlative analysis demonstrated 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% versus time profile up to Month 24. No relevant dose-response relationship was identified for HbF% and clinical efficacy (VF12). For a range of factors explored, no clinically relevant effects of intrinsic, extrinsic, or manufacturing factors were observed for HbF%.

Overall, dose-response and correlative assessment did not identify exa-cel dose as a factor affecting HbF% or clinical efficacy (VF12) based on the limited clinical data. The product allelic editing and % net increase in γ-globin expression appears to correlate with in vivo persistence of gene edited cells. However, the available data don't allow for deriving a threshold of in vivo persistence that correlates with HbF (%) or VF12. The recommended minimum single intravenous dose (3.0×10^6 CD34+ cells/kg) of exa-cel for treatment of SCD was deemed to be acceptable. Please see Clinical Pharmacology review memorandum for further details.

4.4.3 Human Pharmacokinetics

Please see Clinical Pharmacology review memorandum.

4.5 Statistical

Please see the statistics review memorandum.

4.6 Pharmacovigilance

A comprehensive Pharmacovigilance Plan was reviewed by experts in the Office of Biostatistics and Pharmacovigilance (OBPV). The Applicant is already conducting the ongoing long-term follow-up study in the premarket clinical trial setting (Study 131).

To further characterize the serious risk of secondary malignancies and off-target effects of genome editing, under Section 505(o) of Federal Food, Drug, and Cosmetic Act, a safety-related PMR study would be required. The FDA Guidance

Long Term Follow-up After Administration of Human Gene Therapy Products (January 2020) recommends 15-year long-term follow-up for products of gene editing. Please see OBPV review memorandum for details.

5. Sources of Clinical Data and Other Information Considered in the Review

5.1 Review Strategy

The review focused on efficacy data derived from the ongoing Phase 1/2/3 Study 121. All subjects were encouraged to enroll into the long-term follow-up study, Study 131. Therefore, any relevant safety and efficacy information from Study 131 has been incorporated into this review memorandum.

5.2 BLA/IND Documents That Serve as the Basis for the Clinical Review

The review focused on Modules 1, 2 and 5. This included clinical study reports for Studies 121 and 131, along with case report forms and data submitted in response to numerous information requests. Primary efficacy analyses were verified, and other analyses were performed by the review team using JMP 16 software.

The clinical review was primarily based on the Phase1/2/3 Study 121 and long-term follow-up Study 131, with an efficacy data cutoff of February 10, 2023. The protocols are described in Section 6.1 and 6.2, respectively. The team also reviewed relevant documents including pre-BLA review memos, meeting summaries, and study protocols. The team performed efficacy analysis on data submitted from 44 dosed subjects (30 having at least 16 months of follow-up) whose results were submitted in the 90-day safety update with a data lock date of June 14, 2023.

5.3 Table of Studies/Clinical Trials

Data were obtained from Study CTX-001-**121** (NCT03745287) and an open-label long-term follow-up Study CTX-001-**131** (NCT04208529):

- 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.

5.4 Consultations

5.4.1 Advisory Committee Meeting

The 76th Cellular, Tissue, and Gene Therapies AC meeting was convened on October 31, 2023, as the team determined outside expert opinion about the adequacy of the off-target editing analysis was necessary. Appropriate experts

provided discussion on this matter, and the Agency gained insight from passionate patients and patient advocates. The committee ultimately agreed with the Applicant's approach to off-target genome editing evaluation and management, and agreed to the long-term safety studies spanning 15 years.

5.4.2 External Consults/Collaborations

Not Applicable.

5.5 Literature Reviewed

Centers for Disease Control and Prevention, 2023, Data & Statistics on Sickle Cell Disease, accessed August 1, 2023, https://www.cdc.gov/ncbddd/sicklecell/data.html.

Chang, KH, SE Smith, T Sullivan, K Chen, Q Zhou, JA West, M Liu, Y Liu, BF Vieira, C Sun, VP Hong, M Zhang, X Yang, A Reik, FD Urnov, EJ Rebar, MC Holmes, O Danos, H Jiang, and S Tan, 2017, Long-Term Engraftment and Fetal Globin Induction upon BCL11A Gene Editing in Bone-Marrow-Derived CD34(+) Hematopoietic Stem and Progenitor Cells, Mol Ther Methods Clin Dev, 4:137-148.

Cornell, RF, P Hari, and WR Drobyski, 2015, Engraftment Syndrome after Autologous Stem Cell Transplantation: An Update Unifying the Definition and Management Approach, Biol Blood Marrow Transplant, 21(12):2061-2068.

Esteghamat, F, N Gillemans, I Bilic, E van den Akker, I Cantù, T van Gent, U Klingmüller, K van Lom, M von Lindern, F Grosveld, T Bryn van Dijk, M Busslinger, and S Philipsen, 2013, Erythropoiesis and globin switching in compound Klf1::Bcl11a mutant mice, Blood, 121(13):2553-2562.

Hsieh, MM, M Bonner, FJ Pierciey, N Uchida, J Rottman, L Demopoulos, M Schmidt, J Kanter, MC Walters, AA Thompson, M Asmal, and JF Tisdale, 2020, Myelodysplastic syndrome unrelated to lentiviral vector in a patient treated with gene therapy for sickle cell disease, Blood Adv, 4(9):2058-2063.

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Johnson, KM, B Jiao, SD Ramsey, MA Bender, B Devine, and A Basu, 2023, Lifetime medical costs attributable to sickle cell disease among nonelderly individuals with commercial insurance, Blood Adv, 7(3):365-374.

Jones, RJ and MR DeBaun, 2021, Leukemia after gene therapy for sickle cell disease: insertional mutagenesis, busulfan, both, or neither, Blood, 138(11):942-947.

Luc, S, J Huang, JL McEldoon, E Somuncular, D Li, C Rhodes, S Mamoor, S Hou, J Xu, and SH Orkin, 2016, Bcl11a Deficiency Leads to Hematopoietic Stem Cell Defects with an Aging-like Phenotype, Cell Rep, 16(12):3181-3194.

Philippidis, A, 2021, After Analysis, Bluebird Bio Says Vector “Very Unlikely” Cause of Acute Myeloid Leukemia, Human Gene Therapy, 32(7-8):332-334.

Rouillard, AD, GW Gundersen, NF Fernandez, Z Wang, CD Monteiro, MG McDermott, and A Ma'ayan, 2016, The harmonizome: a collection of processed datasets gathered to serve and mine knowledge about genes and proteins, Database (Oxford), 2016

Scott, RB, 1970, Health care priority and sickle cell anemia, Jama, 214(4):731-734.

Steinberg, MH, DH Chui, GJ Dover, P Sebastiani, and A Alsultan, 2014, Fetal hemoglobin in sickle cell anemia: a glass half full?, Blood, 123(4):481-485.

Wardle, SP, CW Yoxall, E Crawley, and AM Weindling, 1998, Peripheral Oxygenation and Anemia in Preterm Babies, Pediatric Research, 44(1):125-131.

Weniger, MA, K Pulford, S Gesk, S Ehrlich, AH Banham, L Lyne, JI Martin-Subero, R Siebert, MJ Dyer, P Möller, and TF Barth, 2006, Gains of the proto-oncogene BCL11A and nuclear accumulation of BCL11A(XL) protein are frequent in primary mediastinal B-cell lymphoma, Leukemia, 20(10):1880-1882.

Williams, TN and SL Thein, 2018, Sickle Cell Anemia and Its Phenotypes, Annu Rev Genomics Hum Genet, 19:113-147.

Yin, J, X Xie, Y Ye, L Wang, and F Che, 2019, BCL11A: a potential diagnostic biomarker and therapeutic target in human diseases, Biosci Rep, 39(11)

Yu, Y, J Wang, W Khaled, S Burke, P Li, X Chen, W Yang, NA Jenkins, NG Copeland, S Zhang, and P Liu, 2012, Bcl11a is essential for lymphoid development and negatively regulates p53, J Exp Med, 209(13):2467-2483.

6. Discussion of Individual Studies/Clinical Trials

6.1 Study 121

6.1.1 Objectives (Primary, Secondary, etc.)

Primary

- Evaluate the safety and efficacy of a single dose of exa-cel in subjects with severe SCD

Secondary

- Assess the effects of infusion of exa-cel on disease-specific events and clinical status; quantify gene editing efficiency

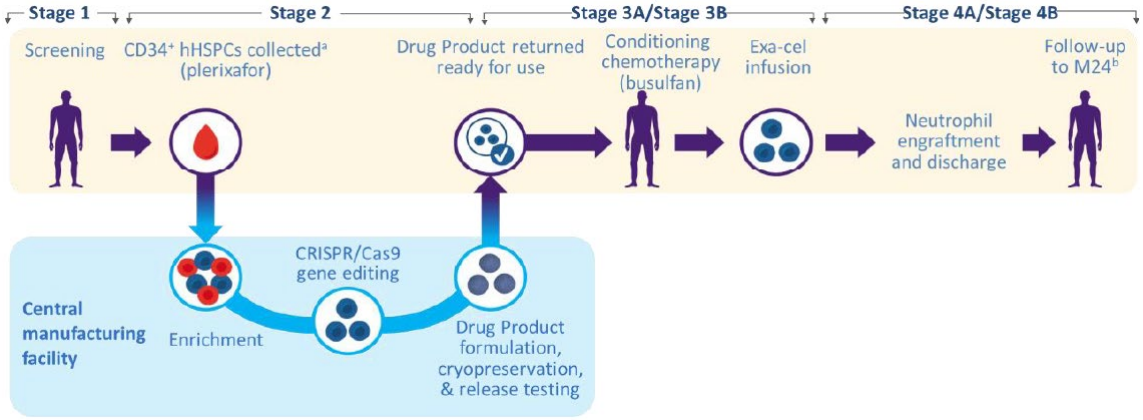
Exploratory

- Assess the ability of biomarkers to characterize exa-cel effect and predict treatment outcomes

6.1.2 Design Overview

Study 121 is a single-arm, open-label, multi-site, single-dose, Phase 1/2/3 study consisting of subjects 12 to 35 years of age with severe SCD. The safety and efficacy of a single dose of exa-cel is being evaluated. The study was conducted in four phases as follows:

Figure 1. Design of Study 121



Source: adapted from study report page 33.
Starting at least 8 weeks before first day of mobilization in Stage 2, subjects received RBC transfusions to maintain HbS level of <30% of total Hb while keeping total Hb concentrations ≤11 g/dL through the start of busulfan conditioning in Stage 3.
[a] Including collection of CD34+ cells as back-up for rescue therapy in the event of non-neutrophil engraftment with exa-cel.
[b] Subjects were followed for approximately 2 years after the exa-cel infusion. All subjects who received exa-cel were asked to enroll into the long-term follow-up study.
Abbreviations: CRISPR/Cas9, clustered regularly interspaced short palindromic repeats-CRISPR associated 9 nuclease; exa-cel, exagamglogene autotemcel; Hb, hemoglobin; HbS, sickle hemoglobin; hHSPCs, human hematopoietic stem and progenitor cells; M24, Month 24.

- Stage 1: Eligible subjects began RBC exchange or simple transfusions for ≥8 weeks before start of mobilization and continued transfusions until they began busulfan conditioning. The goal of the transfusions was to achieve a HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL. Subjects could undergo fertility preservation if desired.
- Stage 2: On Day 1 of mobilization, subjects received plerixafor before apheresis. Apheresis continued for up to 3 consecutive days to collect CD34+ HSPCs. The target CD34+ cell count was ≥15 × 10^6 CD34+ cells/kg for manufacturing of exa-cel in order to achieve a minimum target dose of 3 × 10^6 CD34+ cells/kg. An additional 2 × 10^6 CD34+ cells/kg of backup unedited cells were also obtained.

- Stage 3A: After the exa-cel product was received at the site and the backup CD34+ stem cells were confirmed available, busulfan was started. Busulfan was administered IV through a central venous catheter once daily for 4 consecutive days. Target busulfan area under the curve (AUC) or cumulative exposure for each dosing regimen was the same across all age groups.
- Stage 3B: The exa-cel infusion occurred at least 48 hours and no more than 7 days after completion of the busulfan infusion. On Day 1, the entire dose of exa-cel was infused IV.
- Stage 4A: Subjects were monitored in the transplant unit and received supportive care according to standard practices for subjects undergoing HSCT. Subjects received RBC transfusions to maintain Hb ≥7 g/dL and platelet transfusions to maintain platelets >50,000/µL. Subjects were discharged from the transplant unit upon neutrophil engraftment (absolute neutrophil count ≥500/µL for 3 consecutive measurements on 3 different days) and stabilization of major medical issues, as per local hospital guidelines and investigator judgment.
- Stage 4B: Began after subjects had achieved successful neutrophil engraftment, were clinically stable, and were discharged from the transplant unit. Subjects were followed for the remainder of 2 years after the exa-cel infusion with physical examinations, laboratory and imaging assessments, and adverse event (AE) evaluations.

6.1.3 Population

Key Inclusion Criteria

- Age 12 to 35 (inclusive) as of the date of informed consent
- Documented βS/βS, βS/β0, or βS/β+ genotype with SCD severity characterized by the occurrence of ≥ 2 of the following events per year during the 2-year period before screening, while receiving appropriate supportive care:
    - Acute pain event that requires a visit to a medical facility and administration of pain medications or RBC transfusions
    - Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever
    - Priapism lasting >2 hours and requiring a visit to a medical facility
    - Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL
    - Normal transcranial Doppler (TCD) velocity (time-averaged mean of the maximum velocity <170 cm/sec for non-imaging TCD and <155 cm/sec for imaging TCD) in the middle cerebral artery and the internal carotid artery for subjects 12 to 16 years of age

    - Karnofsky performance status of ≥80% for subjects ≥16 years of age or Lansky performance status of ≥80% for subjects <16 years of age.
- Eligible for autologous transplant

Key Exclusion Criteria

- Willing and healthy 10/10 HLA-matched related donor or prior allogeneic HSCT
- White blood cell (WBC) count <3 × 10^9/L or platelet count <50 × 10^9/L, not related to hypersplenism
- > 10 unplanned hospitalizations or emergency department visits related to SCD in the 1 year before screening that, in the opinion of the investigator, are consistent with significant chronic pain rather than acute pain crises
- HbF level >15.0%, irrespective of concomitant treatment
- Advanced liver disease
- Baseline estimated glomerular filtration rate <60 mL/min/1.73 m^2
- Left Ventricular ejection fraction <45% by echocardiogram
- Lung diffusing capacity for carbon monoxide <50% of predicted value

6.1.4 Study Treatments or Agents Mandated by the Protocol

Conditioning Agent Dosing

The full myeloablative dose of busulfan was stipulated; however, individual sites were permitted to use their preferred approach of daily dosing versus every 6-hour (Q6h) administration.;. Most subjects were treated using Q6h dosing of busulfan which led to a greater percentage of subjects achieving target AUC compared to once-a-day dosing. Busulfan cumulative exposure (AUC) data were analyzed by our clinical pharmacologist. Both busulfan myeloablation doses appeared to be adequate and sufficient, as all subjects with sufficient follow-up of at least 44 days after exa-cel infusion achieved profound neutropenia and engraftment of edited cells, and subsequently had stable allelic editing over time. Please refer to Clinical Pharmacologist's review memorandum for detailed analyses.

Exa-cel Dosing

Exa-cel was administered at a minimum dose of 3 × 10^6 CD34+ cells/kg. This threshold was based on autologous transplantation which typically uses at least 2 × 10^6 to 2.5 × 10^6 CD34+ cells/kg to support engraftment. The maximum cell dose of 20 × 10^6 CD34+ cells/kg was selected based on manufacturing capabilities and projected cell yields at the time of protocol writing. The Applicant, along with the DMC, monitored for any potential dose-related toxicities.

Although every subject was infused per protocol, three received 2.9 x 10^6 CD34+cells/kg. This deviation occurred because the Applicant revised the drug product calculation to account for the density coefficient of the final formulation medium early in the study conduct. Once all doses were recalculated, including for subjects who had already been dosed, three subjects were discovered to have received 2.9 × 10^6 CD34+ cells/kg. According to the Applicant's analysis, the affected subjects' neutrophil and platelet engraftment times and clinical benefit were indistinguishable from those who received the protocol-defined dose.

Reviewer comment:
The dose of exa-cel was chosen based on empiric evidence from the literature of minimal autologous HSPC doses needed to ensure successful engraftment. Although three subjects received slightly less than the protocol dose due to the revision in product calculation, this appeared to be without clinical sequelae, leading to full myeloablation and reconstitution of hematopoietic cells and HbF production, comparable to that of other subjects.

6.1.5 Directions for Use

Prospective patients should be evaluated for overall fitness to undergo HSC transplantation, and be screened for HIV-1, HIV-2, HBV and HCV, and any other infectious agents in accordance with local guidelines.

Mobilization of HSPCs with single-agent plerixafor is followed by apheresis to isolate the CD34+ cells needed for manufacturing. Eight weeks prior to apheresis, patients are required to start a regimen of RBC transfusion with a goal to maintain HbS levels <30% of total Hb while keeping total Hb concentration ≤11 g/dL. Disease-modifying therapies (e.g., hydroxyurea, crizanlizumab, voxelotor) should be stopped 8 weeks before the planned start of mobilization and conditioning. A back-up collection of ≥2 × 10^6 CD34+ cells/kg is required. These unmodified cells must be collected from the patient and be cryopreserved prior to myeloablative conditioning and infusion with exa-cel.

Full myeloablation with busulfan intravenously is necessary before exa-cel infusion.

Any iron chelation should be stopped at least 7 days prior to conditioning, and the clinical site must confirm availability of the complete set of vials comprising the total dose of exa-cel and unmodified rescue cells and inspect the vial(s) for any breaks or cracks.

Anti-seizure prophylaxis with agents other than phenytoin and prophylaxis for hepatic veno-occlusive liver disease (VOD)/hepatic sinusoidal obstruction syndrome should be given prior to initiating busulfan conditioning. Exa-cel must be administered between 48 hours and 7 days after the last dose of the myeloablative conditioning.

Exa-cel should be stored in vapor phase of liquid nitrogen at ≤ -135°C until ready for thaw and administration, then it should be thawed and infused one vial at a time. Patient identity must be confirmed to match the patient information on exa-cel vial(s) before thaw. Patients should be given prophylaxis with an antipyretic and an antihistamine prior to exa-cel. Exa-cel vial(s) need inspection for any breaks or cracks prior to and after thawing, and more than one vial might be needed to deliver dose. Supplies needed to thaw and withdraw the product from the vial(s) will need to be prepared. Exa-cel vials should be thawed at 37°C using a water bath for up to 10 to 15 minutes, until ice crystals are no longer visible in the vial. The thawed exa-cel should appear as a translucent cellular suspension, which may contain visible particles, and should be infused within 20 minutes of thaw.

The entire volume of each vial provided should be infused. If more than one vial is provided, each vial needs to be completely infused before proceeding to thaw and infuse the next vial. Infusion proceeds after vial adapter and filter are attached and the septum is cleaned with an alcohol swab. With the thumb and forefinger of both hands, adapter must be pushed into the vial septum, applying equal pressure until there is a single pop. Exa-cel will be withdrawn into an empty 30 mL syringe connected to the filter. An empty 10 mL syringe will be used to inject 10 mL of saline into the exa-cel vial. Then, the product-filled syringe will be connected to the filter. Exa-cel will be infused through the central venous catheter within 20 minutes of product thaw, after a two-person confirmation and verification of patient’s identification prior to the infusion of each vial(s). Exa-cel should be administered as an intravenous bolus (IV push) and after each vial is infused, the primary line should be flushed with 0.9% sodium chloride solution.

6.1.6 Sites and Centers

While Study 121 was conducted at 16 sites in the United States, Canada, United Kingdom, France, Belgium, Germany, and Italy, the bulk of study data originated from a single U.S. center.

6.1.7 Surveillance/Monitoring

Table 2. Schedule of Activities Starting After Exa-cel Infusion, Study 121

Event/ Assessment	Daily From Day 2 Until Discharge	Follow-up[a,b]													Comments
		D+30[c]	D+60	D+90	D+120	D+150	D+180	D+270	D+360	D+450	D+540	D+630	D+720		
		M1 (±4d)	M2 (±7d)	M3 (±7d)	M4 (±7d)	M5 (±7d)	M6 (±14d)	M9 (±14d)	M12 (±14d)	M15 (±14d)	M18 (±14d)	M21 (±14d)	M24 (±14d)	ETF[cd]	
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Includes blood pressure (systolic and diastolic), temperature, pulse rate, respiration rate, and pulse oximetry. (Section 11.12.3)
Abbreviated physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Section 11.12.3
Performance status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Section 11.12.3
Post CTX001 infusion transfusion regimen							X								Refer to Table 3-5
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Section 11.12.2
Serum chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Section 11.12.2
Coagulation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Section 11.12.2
Hemolysis markers		X		X			X	X	X	X	X	X	X	X	Hemolysis markers include haptoglobin and lactate dehydrogenase (Section 11.12.2)
Immunological testing				X			X		X				X	X	Section 11.12.2
Blood allelic editing (central laboratory)		X	X	X	X	X	X	X	X		X		X	X	Before scheduled transfusion (if applicable) (Section 11.5)
Hemoglobin fractionation (central laboratory)		X	X	X	X	X	X	X	X	X	X	X	X	X	Before scheduled transfusion (if applicable) (Section 11.5)
HbF distribution, F-cells (central laboratory)		X	X	X	X	X	X	X	X	X	X	X	X	X	Before scheduled transfusion (if applicable) (Section 11.5)
Inflammatory and endothelial activation markers (central laboratory)				X			X		X		X		X	X	Before scheduled transfusion (if applicable) (Section 11.5)

Event/ Assessment	Daily From Day 2 Until Discharge	Follow-up[a,b]													Comments
		D+30[c]	D+60	D+90	D+120	D+150	D+180	D+270	D+360	D+450	D+540	D+630	D+720		
		M1 (±4d)	M2 (±7d)	M3 (±7d)	M4 (±7d)	M5 (±7d)	M6 (±14d)	M9 (±14d)	M12 (±14d)	M15 (±14d)	M18 (±14d)	M21 (±14d)	M24 (±14d)	ETF[cd]	
Exploratory biomarker blood samples (central laboratory)				X			X		X		X		X	X	Before scheduled transfusion (if applicable) (Section 11.5.1)
Bone marrow aspirate (central laboratory)							X		X				X	X	For assessment of allelic editing, and exploratory biomarkers (Section 11.7)
DLco [corrected]									X				X	X	Section 11.3
Echocardiograph		X							X		X		X	X	TRV, LVEF, and E/e' assessment (Section 11.4)
12-lead ECG									X				X	X	Section 11.12.5
PRO assessments For ≥18 years of age: EQ-5D-5L, FACT-BMT, and ASCQ-Me For <18 years of age): EQ-5D-Y, PedsQL, and PedsQL SCD module				X			X		X		X		X	X	Assessment should be performed as the first assessment at any visit if possible (Section 11.9) ASCQ-Me will include the following 7 questionnaires: ASCQ-Me SCD Medical History Checklist; ASCQ-Me V 2.0_Sleep Impact Short Form; ASCQ-Me Short Form 2.0_Pain Impact; ASCQ-Me SCD Pain Episode Frequency Severity; ASCQ-Me Short Form 2.0_Social Functioning Impact; ASCQ-Me Short Form 2.0_Emotional Impact; ASCQ-Me Short Form 2.0_Stiffness Impact
PRO assessments (Pain-Scale [11-point NRS])				X	X	X	X	X	X	X	X	X	X	X	Assessment should be performed as the first assessment at any visit if possible (Section 11.9)
Adverse event collection	Continuous from signing ICF and assent (where applicable)														

Event/ Assessment	Daily From Day 2 Until Discharge	Follow-up[a,b]												Comments
		D+30[c]	D+60	D+90	D+120	D+150	D+180	D+270	D+360	D+450	D+540	D+630	D+720	
		M1 (±4d)	M2 (±7d)	M3 (±7d)	M4 (±7d)	M5 (±7d)	M6 (±14d)	M9 (±14d)	M12 (±14d)	M15 (±14d)	M18 (±14d)	M21 (±14d)	M24 (±14d)	ETF[cd]
Prior and concomitant medication	Continuous from signing ICF and assent (where applicable)													

[a] Assessments may be performed over multiple days of visits.
[b] In addition to study-related visits, subjects will be followed per institutional guidelines or as deemed appropriate by the investigator.
[c] Month (M) is defined as 30 days. If Day 30/M1 occurs while the subject is still hospitalized, the daily assessments required before discharge should be done in addition to the Day 30/M1 assessments.
[d] Early Termination of Follow-Up (ETF). Assessments performed within 2 weeks of ETF Visit should not be repeated. Echocardiograph, DLco, and bone marrow aspirate should not be repeated if performed within 6 months of ETF Visit.

Source: Protocol for Study 121 Page 17-19

Reviewer Comment:
The chosen schedule of activities should adequately follow and collect efficacy and safety outcome data over the conduct of the study for all subjects. Generally, subjects had monthly study visits for the first 6 months, and then every 3 months thereafter until Month 24.

6.1.8 Endpoints and Criteria for Study Success

Primary Efficacy Endpoint

- Proportion of subjects who have not experienced any severe VOC for at least 12 consecutive months (VF12) after CTX001 infusion. The evaluation of VF12 starts 60 days after last RBC transfusion needed for post-transplant support or SCD disease management

Key Secondary Efficacy Endpoints

- Proportion of subjects free from inpatient hospitalization for severe VOCs sustained for at least 12 months (HF12) after CTX001 infusion. The evaluation of HF12 starts 60 days after last RBC transfusion needed for post-transplant support or SCD disease management.
- Duration of severe VOC free in subjects who have achieved VF12
- Proportion of subjects with sustained HbF ≥20% at the time of analysis for at least 3 months, 6 months, or 12 months. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Change in number of units of RBCs transfused for SCD over time
- HbF and Hb concentrations over time

6.1.9 Statistical Considerations & Statistical Analysis Plan

Applicant submitted statistical analysis plan (SAP) Version 5.1; the BLA was submitted with results of interim analysis 2 (IA2) based on the data cutoff date of February 10, 2023. However, the review team considered and analyzed subsequently submitted data with a data lock date of June 14, 2023, which boosted the efficacy evaluable population from N=20 to N=30, and the efficacy evaluable adolescents from N=3 to N=6. Please see Statistics review memo for details. However, this subsequent submission was considered IA3 from a

statistical point of view, which would affect statistical analyses, for example, confidence intervals.

The primary efficacy endpoint was the proportion of subjects who achieved VF12, and this was analyzed with a 1-sided P-value against a 50% response rate and a 2-sided 95% exact Clopper-Pearson CI [in the IA2]. The key secondary efficacy endpoint was the proportion of subjects who achieved HF12.

According to the SAP, multiplicity was considered with respect to testing the null hypothesis for the primary and key secondary efficacy endpoints across IAs and the final analysis. The familywise type I error rate would be controlled by an alpha spending approach for tests at interim and final analyses and sequential testing of the primary and key secondary efficacy endpoints (i.e., the key secondary efficacy endpoint will be tested only if the primary efficacy endpoint has crossed an efficacy boundary).

Safety analyses were conducted based on the safety analysis set, unless otherwise specified. Subgroup analyses of age, sex, region, race, and genotype for selected AE summaries were also provided. All safety endpoints were listed by subject.

Reviewer Comment:
Considering the autologous HSPC nature of exa-cel, and the required myeloablative step immediately preceding infusion of exa-cel, a controlled arm study design was not ethical. Therefore, subjects' baseline rate of sVOCs, Hb and HbF levels served as the control. The primary efficacy endpoint is based on the duration free from sVOCs. This parameter is challenging to study as it is a time to event metric, which frequently is based on reporting of pain by the subject (subjective). Subjects were stipulated to have at least 2 sVOCs/year during each of 2 years before screening. With such a severe phenotype, it was anticipated that a difference in baseline vs. post exa-cel rate of sVOCs would be discernable within a study period of practical length. The Applicant chose an efficacy endpoint evaluation period in Study 121, VF12, of freedom from any sVOCs for any period of 12 or more months after the 60-day washout period, following any last RBC transfusion after exa-cel before month 24. This approach, as pointed out by the statistician, increases the likelihood of meeting study success compared to using a fixed 12-month sVOC-free observation period pegged to the time when exa-cel therapy is predicted to start taking effect, such as Month 6 to Month 18. The FDA statistician calculated that this flexibility in the evaluation period could increase the chance of observing a response when there is no treatment effect, compared to a fixed period, by 2 to 3-fold. Please see statistics review memo for details. While sVOCs reported on study underwent blinded adjudication by the Endpoint Adjudication Committee (EAC), the pain adverse events reported on study were evaluated by investigators and then submitted to the EAC. This filtering of pain AEs for submission for adjudication could bias outcomes and is further discussed in the Efficacy Analyses in Section 6.1.11.

6.1.10 Study Population and Disposition

6.1.10.1 Populations Enrolled/Analyzed

The Applicant defined the following analysis populations:

- *Enrolled set:* All enrolled subjects who signed informed consent and met the eligibility criteria.
- *Safety analysis set*: Subset of the enrolled set that included subjects who started the mobilization regimen.
- *Full analysis set (FAS):* Subset of the enrolled set that included subjects who received exa-cel infusion.
- *Primary efficacy set (PES):* Subset of the FAS that included all subjects who were followed for at least 16 months after exa-cel infusion and for at least 14 months after completion of the RBC transfusions for post-transplant support or SCD management. Completion of the (initial) RBC transfusions was determined when all transfusions for post-transplant support or SCD management were finished, followed by 60 days without transfusion. Subjects who completed the 24 months of follow-up in the study after exa-cel infusion were included in this set. In addition, subjects who died or discontinued the study due to AEs considered related to exa-cel and had less than 16 months follow-up after exa-cel infusion, or continuously received RBC transfusions for more than 10 months after exa-cel infusion, were also included in this set. One subject with less than 16 months of follow-up, but determined to be incapable of achieving VF12 responder status when eligible for the PES, was also included.

<u>Reviewer Comment</u>:
The review team analyzed the safety analysis set to assess safety impact of mobilization. However, the primary analysis of safety of exa-cel accounted for the safety events related to administration of exa-cel immediately following full myeloablation, and therefore these analyses were performed on the FAS.

Analysis of efficacy was performed on the PES, who were the subjects with at least 16 months of follow-up after exa-cel which should provide for at least 14 months post-RBC transfusion as of the time of the data lock.

The review team, along with clinical statisticians, were not in agreement with the inclusion of attribution of the cause of death with regard to exclusion of subjects from the denominator of the PES. In addition, one subject failed to meet criterion for success of the primary efficacy endpoint on Study 121 despite having accrued <16 months of follow-up. While the Applicant proposed excluding this subject from the PES because of insufficient follow-up, the FDA review team agreed with the statisticians to redefine the PES to include this subject.

6.1.10.1.1 Demographics

The BLA was submitted with a data lock date of January 10, 2023, following the IA2. The PES N=20 was composed of 10 females (50%). The median (range) age was 21.5 (12 to 34) years, with 3 (15%) subjects ≥12 and <18 years of age. The majority of subjects were Black or African American (95.0%). Subjects were balanced by sex. The baseline median (range) rate of VOCs per year was 3.5 (2.0 to 9.5), with a baseline median (range) rate of hospitalizations for VOCs per year of 2.3 (0.5 to 8.5) and a median (range) annualized duration of hospitalizations for VOCs of 13.0 (2.0 to 64.6) days over 2 years prior to screening. All but 1 subject in the PES were Black or African American (95%), and all subjects had the βS/βS genotype.

At the time of 90-day safety follow-up, with a data lock date of June 14, 2023, data on additional subjects were submitted and analyzed, increasing the FAS to N=44 and the PES to N=30. Among the 44 subjects in the updated FAS, the mean (range) age was 21.2 (12 to 34) years, with 12 (27.3%) subjects ≥12 to <18 years of age. Most subjects (86.4%) were Black or African American. Subjects were approximately balanced by sex.

6.1.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

The IA2 data submitted with the BLA, with a data lock date January 10, 2023, contained N=42 dosed subjects. Of 42 subjects in the FAS, 38 (90.5%) subjects had βS/βS genotype. The baseline median (range) annualized rate of VOCs was 3.5 (2.0 to 18.5) per year, with a baseline median (range) annualized rate of hospitalizations for VOCs of 2.5 (0.5 to 9.5) per year and median (range) annualized duration of hospitalizations for VOCs of 14.0 (2.0 to 136.5) days over the prior 2 years before screening.

In the final data set, with a date of data lock of June 14, 2023, a total of 44 subjects had been dosed. Forty of the 44 subjects in the FAS (90.9%) had βS/βS genotype. The subjects had a mean (range) historical annualized rate of sVOCs of 4.1 (2.0 to 18.5), with a mean (range) historical annualized hospitalization rate for sVOCs of 2.7 (0.5 to 9.5) and a mean (range) annualized duration of sVOC-hospitalization of 19.7 (2.0 to 136.5) days. Subjects had a mean (range) of 11.3 (0 to 86.1) annualized units of RBCs transfused for SCD.

Determination and Adjudication of Baseline sVOCs

Investigators participating in Study 121 were trained in the VOC definition and adjudication process. Subjects' medical records underwent a retrospective search to establish their historical, annualized rate of sVOCs over a 2-year period immediately prior to screening. Events gleaned from the records were submitted to the EAC for adjudication and then used to determine subject eligibility. Among 44 subjects within the FAS, 21 (48%) had fewer than 3 annualized sVOC during the 2-year baseline period. Although the annualized sVOCs should statistically occur approximately every 120 days in a subject with 3 sVOCs/year, there can be seasonal variability since the events might be provoked by common infections

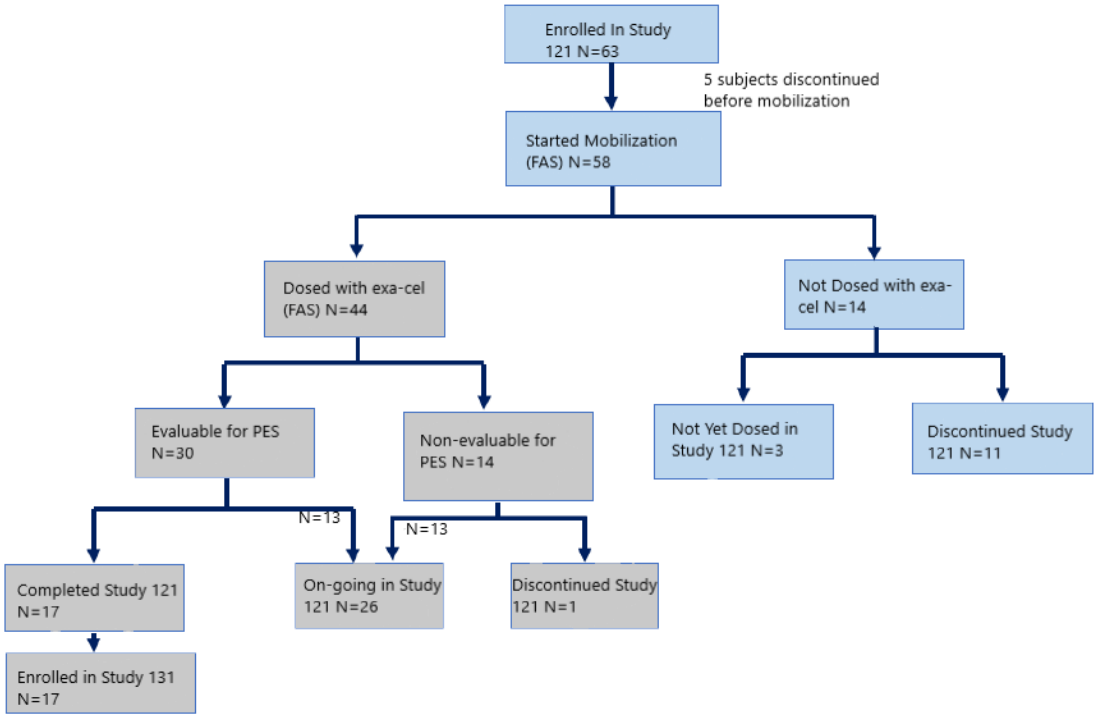
or even cold temperatures. In practice, VOCs may stochastically cluster together, separated by long intervals. It is important to evaluate the specific medical information especially in subjects who have relatively few sVOCs (i.e., milder phenotype) since it could lead to potential inclusion of ineligible subjects (with fewer than 2 annualized sVOCs), potentially overestimating baseline severity and inflating study success. The clinical reviewer reviewed medical information on historical sVOCs, focusing especially on subjects who had the fewest baseline sVOCs and cases where the sVOCs clustered close together and, therefore, might have represented a single sVOC instance.

Reviewer Comment:
The review team perused medical record excerpts which detailed specific medical facility visits for evaluation and treatment of VOC-like events. While, in some cases, the quality of documentation by medical personnel is suboptimal, the review team agreed with the conclusions of the EAC for each of the VOC-like events.

6.1.10.1.3 Subject Disposition

Of 63 subjects enrolled, 58 started mobilization, 44 received exa-cel, and 15 discontinued before receiving exa-cel. Five subjects discontinued before mobilization and 11 subjects after start of mobilization. Of these 11 subjects, 1 was no longer eligible due to kidney function, 1 discontinued due to noncompliance, 1 due to psychological stress, and 2 withdrew consent. Six (10%) subjects failed harvesting of sufficient cells for manufacture of product. One subject dropped out of the study after exa-cel infusion due to death, unrelated to exa-cel Figure 2 shows disposition at time of 90-day safety update.

Figure 2. Subject Disposition, Study 121, Data Lock Date 14 June 2023



Source: Adapted from figure on page 11 of Exa-cel SCD Clinical Overview Addendum: Efficacy and Safety Update 14 June 2023
Abbreviations: FAS, full analysis set; N, number of subjects in the specified group, or the total sample; PES, primary efficacy set.

Reviewer Comment:
Patients with SCD relied on single agent plerixafor for mobilization of HSPCs, due to contraindication of granulocyte-colony stimulating factor (G-CSF) in this population. Analysis of study population disposition is notable for the high prevalence of manufacturing failures (>10%) due to discontinuation for failure to collect adequate CD34+ cells. The mobilization step entails hypertransfusion of RBCs for a period of 8 weeks before start of mobilization (risk of transmitted infections, alloimmunization) and apheresis (requiring insertion of central venous access device), both of which can be associated with adverse events. Furthermore, despite the rigorous RBC transfusions provided during this period, subjects tended to have similar (high) rates of sVOCs during this period. Adequate communication in the USPI is needed to inform potential patients and their clinicians about the possibility of undergoing mobilization/apheresis and yet failing to achieve successful manufacture of exa-cel.

Protocol Deviations

The review team focused on protocol deviations considered to be the most impactful, such as dose deviations and administration of prohibited concomitant medications.

Dose deviations are discussed in Section 6.1.4, study treatments. Regarding administration of prohibited medications, such as agents used to treat sickle cell disease which could confound efficacy findings reported from exa-cel, the team identified only a single subject (b) (6) who took IV crizanlizumab on Day 12. This deviation was unlikely to affect efficacy outcomes mainly due to the early time point shortly after exa-cel, which was still followed by RBC transfusions and a 60-day washout; but also, this was the same subject who failed to reach VF12 because she experienced several sVOCs. One other subject received G-CSF on Day 20, but this was not permitted past Day 21; this is unlikely to have affected efficacy. Lastly, 12 subjects did not sign their informed consent documents on time. In addition, 2 subjects had missing laboratory values related to eligibility.

Other protocol deviations, considered by Applicant as non-important protocol deviations, were likewise reviewed and were concluded to not have substantially biased or impacted study conclusions. These had been submitted in the BIMO Reviewer's Guide and included a variety of deviations such as, for example, minor informed consent document deviations, minor deviations pertaining to laboratory tests or vital signs, timely reporting of serious adverse events (SAEs) to the Applicant by the investigators, or out of window drawing of blood samples.

Reviewer Comment:
Protocol deviations were reviewed and considered by reviewer. The reviewer concluded that they were unlikely to have biased outcomes or to have detracted meaningfully from the interpretability of study results.

6.1.11 Efficacy Analyses

6.1.11.1 Analyses of Primary Endpoint(s)

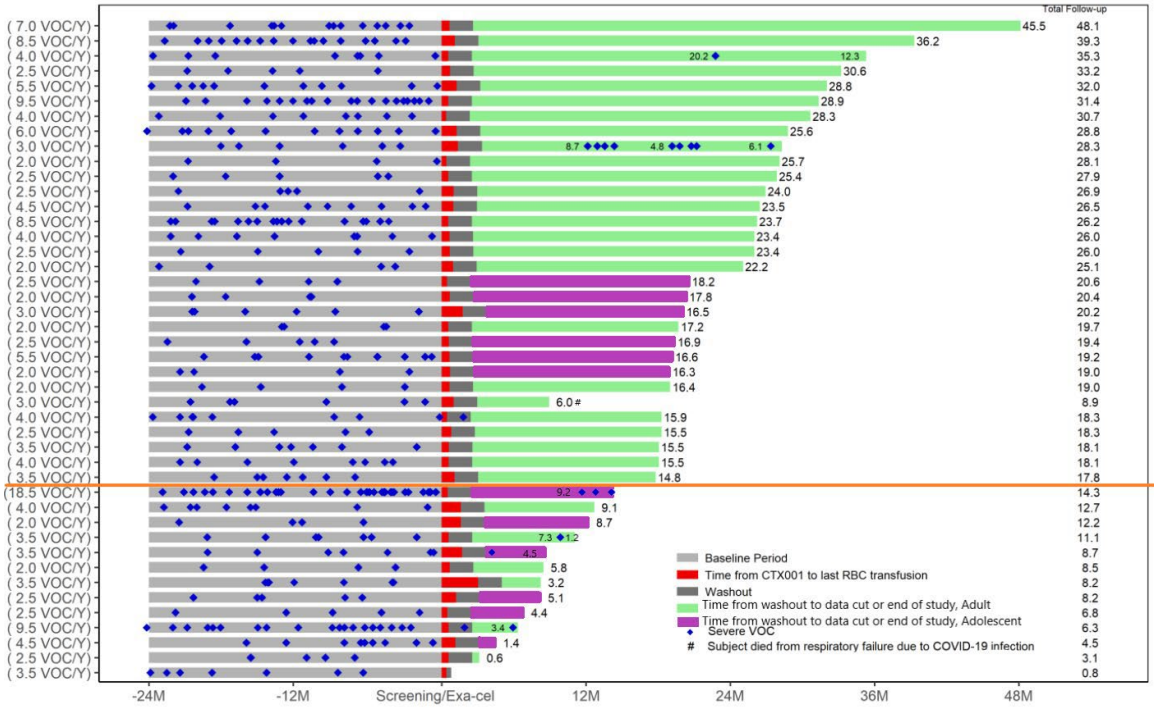
The primary efficacy endpoint was designed to inform of a clinical benefit of reduction or elimination of VOCs, as this manifestation of SCD is the most common and morbid event. As described in the preceding sections, subjects needed to demonstrate a baseline annualized rate of at least two sVOCs for 2 years prior to enrollment. This was determined retrospectively, but the events were submitted to the EAC for adjudication using the same definitions as any sVOCs reported post exa-cel.

Efficacy Analysis

Of 44 dosed subjects at the time of data lock, 30 subjects had follow-up sufficient to be evaluable for efficacy analysis, and 29 (96.7%) achieved the primary efficacy endpoint of VF12. Of the 29 subjects who achieved VF12, 28 remained free of sVOCs for a mean duration of 22.3 (SD 7.2) months, with a maximum of 45.5 months. All 30 (100%) evaluable subjects had achieved HF12, a key secondary efficacy endpoint, indicating absence of SCD related hospitalizations for a period of ≥12 months at any point on study after exa-cel and a ≥60-day

washout from any RBC transfusions for SCD or autologous transplant. Figure 3 depicts sVOCs before and post-exa-cel infusion.

Figure 3. Historical and Post Exa-cel VOCs and sVOC-Free Duration in All Dosed Subjects, Study 121, FAS N=44



Source: Modified from SCD Clinical Overview Addendum: Efficacy and Safety Update 14 June 2023, page 19.
Notes: Only severe VOCs that were adjudicated by the EAC as meeting the protocol criteria were displayed for both the baseline period and the post exa-cel infusion period. Baseline period was the 2 years prior to most recent screening. The number on the right end is the duration of total follow-up in months. (# VOC/Y) on the left end is the baseline annualized rate of severe VOCs. Last RBC transfusion refers to the last RBC transfusion for post-transplant support or SCD management during the initial RBC transfusion period. Orange line indicates 16 months of follow-up.
Abbreviations: CTX001, exa-cel; EAC, Endpoint Adjudication Committee; FAS, Full Analysis Set; PES, Primary Efficacy Set, RBC, red blood cell; SCD, sickle cell disease; VF12, absence of any severe VOCs for at least 12 consecutive months after exa-cel infusion; VOC, vaso-occlusive crisis; Y, year.

Considering that pain events comprised most of the clinical evidence behind the primary efficacy endpoint, and that pain is a subjective experience, the Applicant proposed defining and using sVOCs (instead of just VOCs) for efficacy analysis, due to a lack of adequate corroborative laboratory or radiologic strategies. To be considered severe and included as sVOCs in the analysis, the acute pain events required a visit to a medical facility and administration of pain medications (opioids or IV non-steroidal anti-inflammatory drug [NSAIDs]) or RBC transfusions.

Study 121 investigators determined which pain events were potential sVOCs and forwarded the clinical information to the EAC for independent adjudication.

In addition to pain events, other clinical presentations also could constitute sVOCs, including: acute chest syndrome, indicated by presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever; episodes of priapism lasting >2 hours and requiring a visit to a medical facility;

and splenic sequestration, defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL.

Adjudication of Treatment-Emergent VOC-Like Events

The investigators assessed events that were potential sVOCs and submitted a subset of these to the EAC for adjudication, while events not submitted were reported as AEs. To decrease risk of bias, considering that the initial attribution by the investigator would filter certain events for reporting to the EAC, the investigators were required to provide rationale for VOC-like events occurring ≥60 days after the last RBC transfusion for post-transplant support or SCD management that were not submitted to the EAC. These were identified in a customized MedDRA query by the Applicant.

The review team performed analysis of VOC-like adverse events in the ADAE dataset, including pain events and respiratory events like pneumonia, as well as specific AEs, like cases of priapism, for further scrutiny . From among these AEs, the team selected 13 events from among 9 subjects for detailed review and sent an IR to request narratives and supporting information including physical examination findings, vital signs, Numeric Pain Rating Scale scores, and laboratory values if available at the time of the event.

The review team adjudicated the vast majority of these AEs as non-sVOCs for reasons such as: cases of pneumonia with no infiltrate on radiograph; not meeting criteria for acute chest syndrome; a month-long case of priapism for which the subject did not seek evaluation at healthcare facility; or pain events for which no opioids, IV NSAIDS, or RBC transfusions were required.

A few events were more difficult to evaluate as they comprised pain and were attributed by investigator to the subject's "chronic pain with narcotics dependence" or "drug seeking behavior." In the case of Subject (b) (6) two AEs of grade 3 pain (one an SAE) that required IV narcotics and NSAIDs on Day 683 to 684 and on Day 685 to 686, were, in the opinion of the investigator, not sVOCs because the subject had chronic pain exacerbation and narcotic dependence, and thus were not submitted to the EAC for adjudication. The subject had gone to an emergency department not associated with the study site for evaluation.

Reviewer Comment:
While attribution of the pain AEs, especially in the cases such as subject (b) (6) is difficult, where multiple layers of psycho-social and chronic analgesics dependence might cofound the clinical picture, the reviewer concluded that the AEs in question were not sVOCs, but rather more likely attributable to chronic pain exacerbations.

sVOCs Reported Post Exa-cel Infusion

While most subjects achieved the primary efficacy endpoint and remained free from sVOCs for extended periods, seven did experience sVOCs following exa-

cel. Of these, two sVOC occurred within 60 days of any last RBC transfusion for autologous transplant support or SCD management and thus took place before the start of the efficacy evaluation period. A total of six subjects did experience sVOCs beyond the 60-day washout period after RBC transfusions and thus within the efficacy observation period.

- Subject (b) (6) reported 9 sVOCs between 12.1- and 21.2-months post exa-cel and did not achieve VF12. The subject did have similar pharmacodynamic outcomes to subjects who reported no sVOCs. According to the narrative provided, she presented to various healthcare facilities reporting SCD-like pains but denied VOCs to the clinical site related to study conduct. The surrogate endpoints of HbF and allelic editing of this subject were indistinguishable from those achieved by subjects who attained VF12.
- Subject (b) (6) achieved VF12 and experienced no sVOCs from exa-cel infusion until 20.2 months, when the subject had a sVOC which required hospitalization; further information strongly suggests that the event was triggered by and was related to a parvovirus infection. This subject subsequently went on to accrue 12.3 months more of follow-up without sVOCs as of data lock date.
- Subject (b) (6) experienced 3 sVOCs between 11.7 and 14.1 months after exa-cel, and although had only 14.3 months of follow-up, failed to meet the definition of VF12. The subject presented to an emergency department unaffiliated with the study site with complaints including generalized pain starting in lower back described as “sickle cell like,” body aches, and for some visits, chest pain with shortness of breath. This subject had the highest baseline frequency of sVOCs at 18.5 per year at baseline.

The following subjects experienced sVOCs in the evaluation period but still have the potential to achieve VF12 with further follow-up.

- Subject (b) (6) experienced a sVOC with hospitalization at 9.9 months post exa-cel, with a report of lower extremity pain, which resolved within 24 hours.
- Subject (b) (6) had a sVOC at 4.2 months after exa-cel, presenting with chest pain, lower back pain, and a headache.
- Subject (b) (6) had two sVOCs with hospitalization. The first was within the transfusion washout period on study day 57, while the second event was reported at 5.9 months after exa-cel. This event consisted of lower extremity pain and chest pain immediately after endoscopy, because of which the subject could not take oral analgesics (long-acting opioid for chronic pain) in preparation for endoscopy. Subject was hospitalized for 7 days until oral intake was tolerated (endoscopy confirmed reflux esophagitis with possible eosinophilic gastritis and duodenitis).

Each of the six subjects with adjudicated sVOCs that occurred after the 60-day RBC washout period had no observed difference in pharmacological response to exa-cel, with HbF% increases after exa-cel treatment comparable to other

subjects who had no VOCs, and each had a high and stable percent allelic editing.

Reviewer Comment:
The Applicant's view is that the above post exa-cel pain events adjudicated by the EAC as sVOCs nevertheless may not be related to acute sickling, considering the subjects' robust F cell and HbF parameters, which should be protective, and considering the history of chronic narcotic use and chronic pain in many of the subjects. Due to the subjective nature of pain, adjudication of pain events in these subjects is challenging. There are reports from allo-HSCT for SCD evaluating painful events after engraftment, suggesting a pattern of gradual decrement in VOC event frequency that continues for at least one year post transplant, even with successful engraftment of donor HSPCs.

6.1.11.2 Analyses of Secondary Endpoints

Freedom From Hospitalization for ≥12 Months Post Exa-cel

The key secondary efficacy endpoint was defined as the proportion of subjects achieving HF12, and this was assessed only after a washout from any last RBC transfusion for SCD management or autologous transplant support of ≥60 days. All 30 subjects (100%) with ≥16 months of follow-up achieved HF12.

As of June 14, 2023, 43 of 44 subjects in the FAS had at least 60 days of follow-up after the last RBC transfusion for post-transplant support or SCD management. Of these 43 subjects, 40 subjects were free from inpatient hospitalization for VOCs after exa-cel infusion through the duration of follow-up in Studies 121 and 131 for median (range) of 16.8 (0.6 to 45.5) months, starting 60 days after last RBC transfusion. One subject required hospitalization for likely a parvovirus infection with sVOC at 22.7 months post exa-cel. The two remaining subjects have <16 months of follow-up and required one hospitalization each for sVOCs.

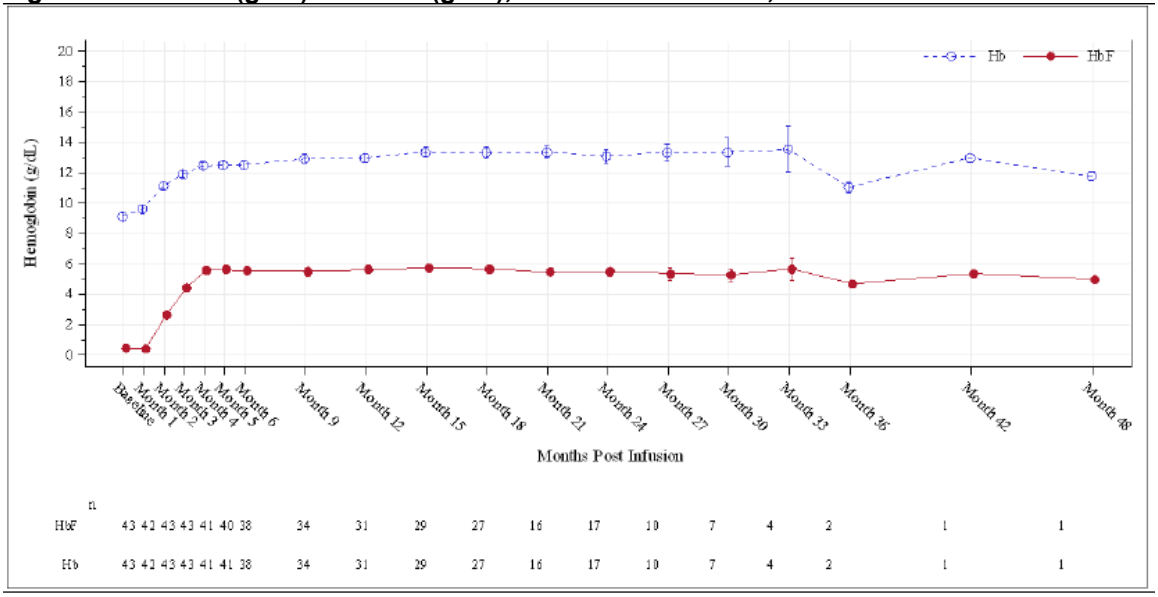
Surrogate Efficacy Endpoints

Levels of HbF typically drop from >80% of total Hb at birth to <1% at 1 year of age. In most adults with sickle cell anemia, HbF levels are increased; however, the magnitude of this increase varies greatly. HbS deoxygenation-induced polymerization is the driver of SCD pathophysiology, leading to RBC deformation and sickling. Fetal hemoglobin inhibits deoxy sickle hemoglobin polymerization, and thus ameliorates SCD phenotype severity. HbF production is restricted to a small number of erythroid precursors; their progeny in the blood are called F-cells. Study 121 excluded those candidates whose HbF was >15% at baseline, and the resulting dosed population had a median HbF of 5%, ranging from 0 to 14.7%.

The increased mean Hb levels and HbF (%) were achieved early (by Month 3) after exa-cel infusion and were generally maintained over time from Month 6 through end of follow-up period. The median HbF% at month six among the 39 subjects who had a level drawn was 44%, ranging from 14.9 to 68.4%. The subject with 14.9% eventually died of respiratory failure and, while on extra corporeal membrane oxygenation (ECMO) in the intensive care unit, was heavily transfused with RBCs, thus diluting HbF. Excluding this subject's level, the next lowest subject had 27.7% HbF at 6 months. The percent change (increase from baseline to 6 months) among the N=38 subjects was 40.5%, ranging from 13.3 to 64.6%. The mean (SD) total Hb levels were 11.9 (1.5) g/dL at Month 3 and were maintained with a mean of ≥11.1 g/dL from Month 6 onward. Please see Figure 4.

The mean (SD) proportion of total Hb composed of HbF was 36.9% (9.0%) at Month 3 and was maintained at generally ≥40% from Month 6 over the duration of follow-up. Please also see the clinical pharmacology review memo for more details.

Figure 4. Total Hb (g/dL) and HbF (g/dL),Studies 121 and 131, FAS



FAS: Full Analysis Set; Hb: hemoglobin; HbF: fetal hemoglobin; n: size of subsample; SCD: sickle cell disease
Notes: Mean values are plotted in the line, mean + SE and mean – SE values are plotted as bars at each visit. The numbers of subjects with total Hb and HbF values available at the corresponding visits are shown at the bottom. Baseline was defined as the most recent non-missing measurement (scheduled or unscheduled) collected before the start of mobilization in Study 121. Analysis visit was used in the figure.

Source: Clinical Overview Addendum: Efficacy and Safety Update 14 June 2023, Page 31.

Proportion of Subjects With Sustained HbF ≥20%

All 30 (100%) subjects in the PES had sustained HbF ≥20% for at least 12 consecutive months starting 60 days after the last RBC transfusion.

Allelic Editing

An important confirmatory parameter underpinning the efficacy of exa-cel is the detection of persistent evidence of allelic editing. In the FAS, allelic editing in the bone marrow (CD34+ cells) and peripheral blood (nucleated cells) remained stable for the duration of follow-up (up to Month 24 [bone marrow] and up to Month 42 [peripheral blood, including Study 121 and 131]). Please see Table 3; note that bone marrows were collected at Months 6, 12, and 24.

Table 3. Proportion of Edited Alleles (%) in Marrow and Peripheral Blood, Studies 121 and 131

Visit Statistic	Bone Marrow (%) N=44	Peripheral Blood (%) N=44
Baseline	--	--
n	--	44
Mean (SD)	--	0.2 (0.1)
Month 3	--	--
n	--	42
Mean (SD)	--	70.9 (10.6)
Month 6	--	--
n	37	38
Mean (SD)	86.1 (7.6)	73.4 (8.1)
Month 12	--	--
n	31	31
Mean (SD)	86.2 (8.6)	74.2 (8.7)
Month 18	--	--
n	--	25
Mean (SD)	--	76.0 (9.1)
Month 24	--	--
n	16	17
Mean (SD)	88.5 (4.6)	79.2 (5.6)

Source: Derived by reviewer from ADAEF2
Abbreviations: N, number of subjects in group; SD, standard deviation

Reviewer Comment:
Although it is known that HbF ameliorates the phenotype of SCD by inhibiting deoxy-HbS polymerization, neither blood HbF concentration, nor the prevalence of F-cells (RBCs with detectable HbF), measures the amount of HbF/F-cell. The best predictor of the likelihood of severe SCD may be the HbF/F-cell, rather than the total number of F-cells or concentration of HbF. It has been reported that even some patients with high HbF can have severe disease because HbF may be unevenly distributed among F-cells, with certain cells having insufficient concentrations to inhibit HbS polymerization. Only when the total HbF concentration is near 30% is it possible for the number of protected cells to approach 70% (Steinberg et al. 2014). Surrogate efficacy parameters from Study 121 provide support of the clinical benefit observed based on primary and secondary efficacy endpoint findings. A large majority of subjects go on to produce a high, sustained concentration of HbF and maintain allelic edits.

Hemolysis Markers

SCD is associated with chronic hemolytic anemia, and thus the Applicant evaluated a number of hemolytic markers in Study 121. Exa-cel treatment generally led to improvement in hemolysis assessments over time, including absolute reticulocyte count, indirect bilirubin, lactate dehydrogenase (LDH), and haptoglobin.

- At baseline, subjects in the PES with available LDH data (N=29) had elevated mean (SD) LDH levels of 474.3 (200) U/L. Following exa-cel treatment, among N=29 subjects, the mean LDH levels normalized with mean (SD) of 239.2 (145) U/L by Month 9 and were generally maintained over time.
- Haptoglobin is frequently absent or diminished in the context of ongoing hemolysis, such as in patients with SCD. At baseline, subjects in the PES (N=29) had low mean (SD) haptoglobin levels of 0.0797 (0.086) g/dL, and haptoglobin was detectable in only 22 of 29 (75.9%) subjects with baseline measurements. Following exa-cel, mean haptoglobin levels became detectable at Month 3 in 27 of 29 (93.1%) subjects with measured values, and remained above baseline throughout follow-up.

Reviewer Comment:
Compared with baseline values, hemolysis markers after exa-cel trended towards normal, indicating diminished hemolysis, which supports the overall efficacy findings.

RBC Transfusion Reduction from Baseline for SCD Related Indication

Among the 44 exa-cel dosed subjects, 38 received at least one RBC transfusion in the 2 years before screening (baseline). None required any RBC transfusions for an SCD indication (100% reduction in RBC transfusion SCD-related indications starting 12 months after exa-cel infusion). One subject did require RBC transfusion on Day 500 due to a gunshot wound and resultant hemorrhage.

Patient-Reported Outcomes Over Time

The Applicant included analysis of patient-reported outcomes (PROs) in Study 121 which, for adults (≥18 years of age), used tools including: 11-point NRS pain scale, FACT-BMT, ASCQ-Me, and EQ-5D-5L. For adolescents <18 years of age the tools used included: 11-point NRS pain scale, PedsQL, PedsQL SCD module, and EQ-5D-Y. Overall, for subjects ≥18 and ≤35 years of age at screening in the PES, PRO scores assessing pain, health and disease status, quality of life, and general well-being improved by Month 6 with a sustained response up to Month 24.

Reviewer Comment:

Although the analysis of the PRO tools over time suggested improvement after exa-cel, a serious weakness of this analysis is the single arm design of Study 121. Without a control arm receiving a different intervention, it is challenging to ascribe changes in PROs to exa-cel, therefore, the review team does not plan to include these outcomes in the USPI.

6.1.11.3 Subpopulation Analyses

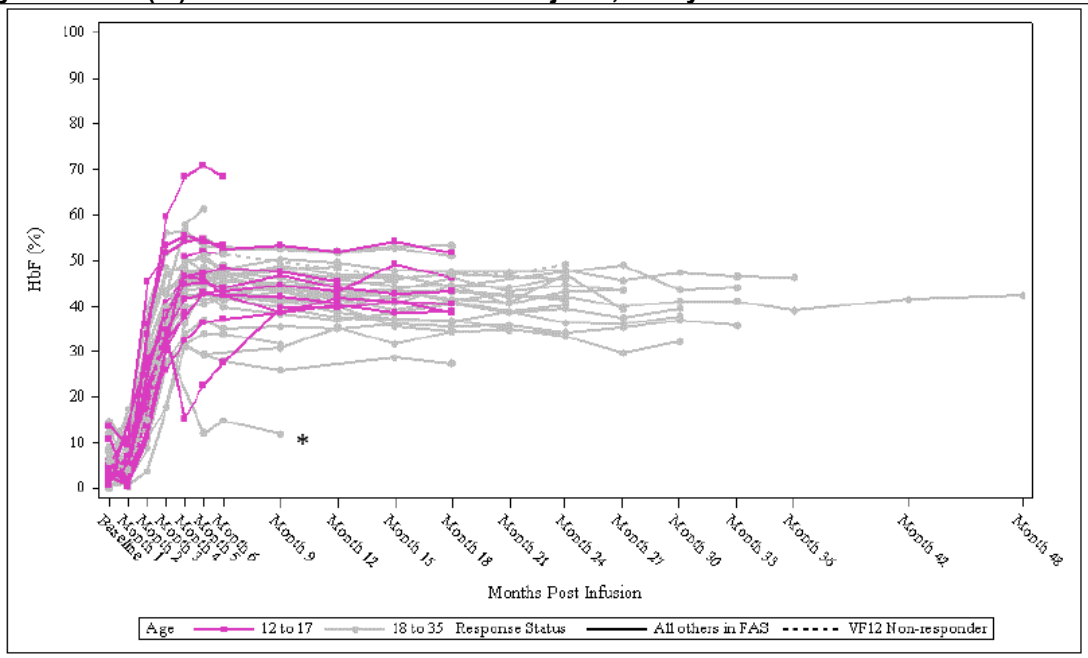
Primary Efficacy Endpoint (VF12) Among Adolescents vs. Adults

All 6 adolescents (100%) included in the PES achieved VF12 and had between 19 and 20.6 months of follow-up. One additional adolescent failed to achieve VF12 by protocol definition but was not included in PES by the Applicant due to a follow-up <16 months. This subject had 14.3 months of follow-up and experienced three sVOCs between Month 11.1 and 14.1 (thus it was impossible for the subject to have reached a 12-month period free of sVOCs on Study 121).

HbF Expression in Adults vs. Adolescents

HbF production among adolescents treated with exa-cel increased and remained overall stable on Study 121. All adolescent subjects (N=12) with available Month 6 HbF (%) data, had HbF (%) levels of ≥20% by Month 6, similar to outcomes in the overall population. Please see Figure 5, which graphs HbF% over time among the FAS population, separating adults from adolescents by color of lines used.

Figure 5. HbF (%)in Adult and Adolescent Subjects, Study 121



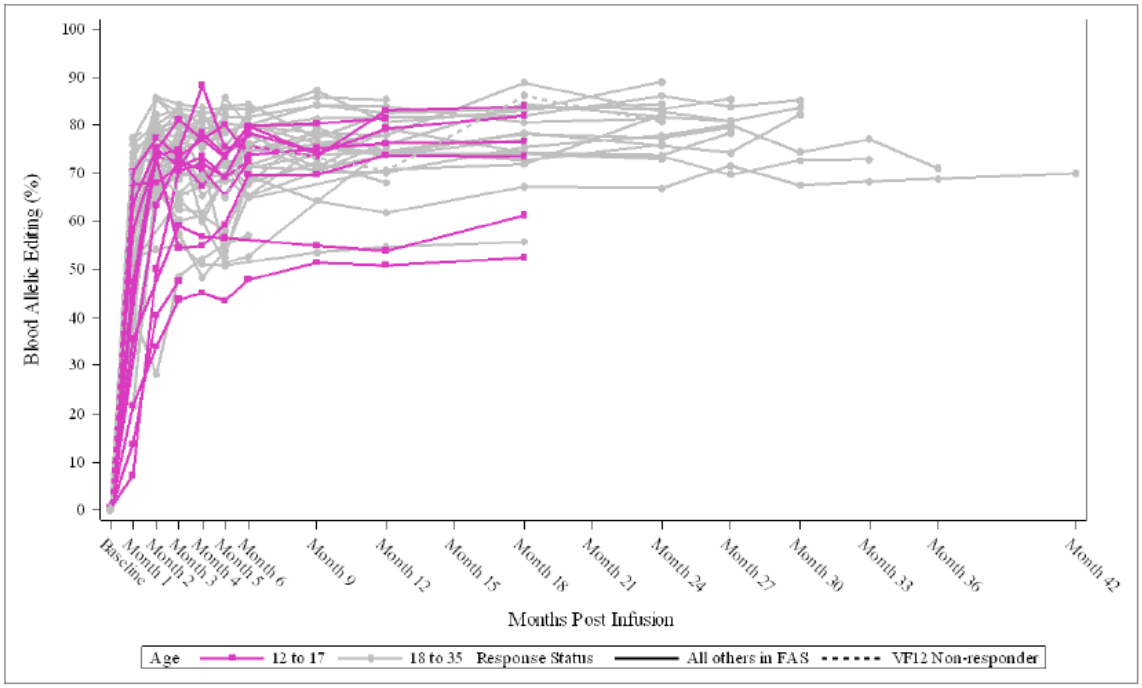
exa-cel: exagamglogene autotemcel; FAS: Full Analysis Set; HbF: fetal hemoglobin; SAE: serious adverse event; SCD: sickle cell disease; VF12: not experienced any (i.e., absence of) severe VOC for at least 12 consecutive months after exa-cel infusion; VOC: vaso-occlusive crisis

Source: Reproduced from Clinical Overview Addendum: Efficacy and Safety Update 14 June 2023, Page 40.
* in the figure indicates subject who required RBC transfusions during terminal hospitalization for respiratory failure resulting in death, and thus had dilution of HbF. In addition, another subject with a dip at Month 4) had an exchange transfusion just before Month 4 for an SAE unrelated to exa-cel or SCD (vision blurred). Baseline was defined as the most recent non-missing measurement (scheduled or unscheduled) collected before the start of mobilization in Study 121. Analysis visit was used in the figure.

Allelic Editing of Adults vs. Adolescents

Expression of allelic editing was stable in adolescent subjects treated with exa-cel, similar to adult outcomes, although the numbers were very small. The peripheral blood nucleated cells allelic editing analysis is shown in Figure 6:

Figure 6. Proportion of Alleles With Genetic Modification in Peripheral Blood of Adults and Adolescents, Study 121



FAS: Full Analysis Set; SCD: sickle cell disease; VF12: not experienced any (i.e., absence of) severe VOC for at least 12 consecutive months after exa-cel infusion; VOC: vaso-occlusive crisis

Note: Baseline was defined as the most recent non-missing measurement (scheduled or unscheduled) collected before the start of mobilization. Analysis visit was used in the figure.

Source: Reproduced from Clinical Overview Addendum: Efficacy and Safety Update 14 June 2023, Page 43

Male vs. Female

Among the N=30 subjects in PES, 14 were female and 16 were male. All 16 male subjects achieved VF12, versus 13 of 14 female subjects.

Genotype

All 14 female subjects in the PES were of homozygous βS genotype, including the subjects who failed to achieve VF12. One male subject had βS/β0 genotype and the remaining 15 male subjects were βS homozygotes.

Reviewer Comment:
There do not appear to be trends suggesting a differential outcome with respect to the primary efficacy endpoint when analyzing subpopulations by sex or genotype. The vast majority of subjects in the PES carried the βS/βS genotype. Considering primary efficacy outcomes by age groups, 23 of 24 (96%) of adults achieved VF12 compared with six of seven (86%) adolescents, whose VF12 could be analyzed at date of data lock, (this includes the adolescent with 14.3 months of follow-up who suffered VOCs between month 11.5 and 14). The difference between the propensity to achieve VF12 among adults and

adolescents is likely due to chance, considering the small numbers of the evaluable adolescent subjects.

6.1.11.4 Dropouts and/or Discontinuations

Only one subject discontinued after infusion of exa-cel due to death and is discussed in detail in the safety section on deaths.

6.1.12 Safety Analyses

Safety data come from Study 121 and the long-term follow-up Study 131.

Safety data submitted to the BLA came from IA2 of Study 121 conducted in subjects aged ≥12 to 35 years with up to 2 years of follow-up after exa-cel infusion, with a cutoff date of February 10, 2023, as well as data from Study 131, a long-term follow-up study. Additional data were analyzed from the 90-day safety update with a cutoff date of June 14, 2023, from Studies 121 and 131. Study 121 safety assessments included AEs, SAEs, transplant-related mortality (within 100 days and 1 year of infusion), all-cause mortality, engraftment (neutrophil and platelet), clinical laboratory assessments, vital signs, 12-lead ECGs, and physical examinations. Study 121 subjects received myeloablative conditioning with busulfan. Exa-cel was dosed at ≥3 × 10^6 CD34+ cells/kg as a single infusion.

6.1.12.1 Methods

Exa-cel therapy consists of harvesting HSCs from the patient for ex vivo gene editing followed by reinfusion of modified HSPCs after myeloablative chemotherapy administration. Consequently, this alkylator chemotherapy exposure may lead to therapy-related myeloid neoplasms from busulfan myeloablation. Busulfan will not affect the transplanted/edited HSCs, but may not be completely myeloablative, theoretically permitting survival of residual HSPCs with chemotherapy-induced mutations. Long-term follow-up to report on incidence of neoplastic adverse events will be conducted on all consenting subjects in Study 131, where they will be followed for 15 years from exa-cel infusion.

The main focus of the safety analysis is treatment-emergent adverse events (TEAEs) reported starting with administration of the investigational product on Day 0. However, the process necessary to manufacture exa-cel entails mobilization of HSPCs from the bone marrow using plerixafor and harvesting of cells via apheresis, followed by myeloablation which is completed 2 to 7 days prior to infusion of exa-cel. This prerequisite process exposes subjects to certain risks incumbent on the administration of plerixafor, insertion of apheresis catheter and the apheresis procedure itself, and administration of busulfan. Therefore, the study's safety analysis population includes subjects who started on mobilization. Consequently, the Agency reviewed the safety of the above process and analyzed TEAEs.

Disposition

Of 63 subjects enrolled, 58 started mobilization, of whom 42 received exa-cel and 15 discontinued before receiving exa-cel. Five subjects discontinued before mobilization and 10 subjects after start of mobilization. Of these, six had inadequate cell harvest, one was no longer eligible due to kidney function, 1 discontinued due to noncompliance and 2 withdrew consent. One subject discontinued after exa-cel infusion due to death.

Safety Analysis During Mobilization and Apheresis

A total of 58 subjects embarked on mobilization with plerixafor. Fifty-seven of these (98.3%) experienced at least 1 AE during the mobilization and apheresis period (before conditioning), although most AEs were non-serious and Grade 1 or 2 severity. Nevertheless, 40 (69%) subjects experienced Grade 3 or 4 AEs.

Fourteen (24.1%; N=58) subjects had AEs that were considered related to plerixafor and 19 (32.8%; N=58) subjects had AEs that were considered possibly related to plerixafor.

Thirty-five (60.3%; N=58) subjects had an SAE. Of these, 8 (13.8%) subjects had at least 1 SAE considered related or possibly related to plerixafor.

The most common AEs during mobilization and apheresis (occurring in >10% of subjects) were nausea, vomiting, headache, abdominal pain, pruritis, vascular access site pain, constipation, pain in extremity, sickle cell anemia with crisis, hypomagnesemia, back pain, diarrhea, pyrexia, hypokalemia, arthralgia, hypocalcemia, pain, paresthesia, fatigue, and oral paresthesia. The majority of these AEs were consistent with the known safety profile of plerixafor, apheresis procedure, or underlying disease.

During the mobilization and apheresis period, but before conditioning, a total of 22 (37.9%; N=58) subjects had an AE of sickle cell anemia with crisis and 4 (6.9%) subjects had an AE of ACS; of those, 3 subjects had AEs of both sickle cell anemia with crisis and ACS.

All VOC events (i.e., sickle cell anemia with crisis and ACS) were Grade 2 or 3 in severity and most were considered not related to plerixafor, having occurred >7 days after any plerixafor dose. Twenty (34.5%; N=58) subjects had an SAE of sickle cell anemia with crisis and 3 (5.2%) subjects had an SAE of ACS; of those, 2 subjects had SAEs of both sickle cell anemia with crisis and ACS. Within 7 days after any plerixafor dose, 9 (15.5%; N=58) subjects had an SAE of sickle cell anemia with crisis and 1 (1.7%) subject had an SAE of ACS.

Reviewer Comment:
While VOCs reported during mobilization and apheresis were a minority of all AEs, most VOCs and ACS events were SAEs. The rate of reported VOCs during mobilization and apheresis was analyzed by the Applicant and, based on response to an IR, the Applicant reported that this rate is similar to the

annualized baseline rate of VOCs experienced by the subjects before study entry. However, this must be considered in context of the aggressive RBC transfusion regimen that the subjects received 8 weeks prior to start of mobilization, diluting their HbS to <30% while keeping total Hb ≤11 g/dL. Considering the low concentration of sickle hemoglobin which the subjects had during this interval, the substantial rate of VOC AEs is puzzling and unexpected. This might be of particular importance to the 10% of subjects who embarked on this journey, regardless of the potential risks associated with the RBC transfusion regimen, mobilization, and apheresis, only to fail to have sufficient cell harvest needed to manufacture product, and thus did not receive exa-cel treatment.

Post Exa-cel Safety Analysis

Study 121 monitored subjects' safety from the beginning of mobilization until the day before infusion of exa-cel, and any events were analyzed and discussed in the section above. This included the administration of busulfan as well as the necessary wash out period, which was defined as 2 to 7 days.

The median exa-cel dose was 4 (2.9 to 14.4) x 10^6 CD34+ cells/kg. The 44 dosed subjects were followed for a median duration of 19.3 (range: 0.8 to 48.1) months (including on long-term follow-up Study 131).

Exposure

Busulfan was administered according to local practice, with once a day or Q6h infusion. For the 42 subjects in the FAS, 28 subjects (66.7%) and 14 subjects (33.3%) received Q6h and once a day regimen of busulfan, respectively. The target busulfan cumulative area under the curve (cAUC) was 74 mg*h/L (range: 59 to 89) for the Q6h regimen and 82 mg*h/L (range: 74 to 90) for the once a day regimen. Twenty-six (93%) of the 28 subjects receiving the Q6h regimen and 9 (64%) of the 14 subjects receiving the once a day regimen were within protocol-specified cAUC target range. The median exa-cel dose was 4.1 (range: 2.9 to 14.4) × 10^6 CD34+ cells/kg.

Reviewer Comment:
The dose of busulfan led to 100% pancytopenia in study subjects, followed by engraftment of hematopoietic cells without the need for use of back-up, unedited cells. This suggests adequate conditioning was delivered. The target exa-cel dose was delivered to all subjects treated, except three who were infused with 2.9 x 10^6 CD34+cells/kg. This deviation occurred because the Applicant revised the drug product calculation early in the conduct of the study to account for the density coefficient of the final formulation medium. Once all doses were recalculated, including for subjects who had already been dosed, three subjects were discovered to have gotten 2.9 × 10^6 CD34+ cells/kg.

6.1.12.2 Overview of Adverse Events

Adverse Events

Exa-cel therapy requires complete myeloablation prior to infusion, and busulfan was administered for this purpose. Most AEs were reported after the start of conditioning and included gastrointestinal toxicities such as emesis and mucositis and myelosuppression (cytopenias). As such events are expected complications of myeloablative conditioning with busulfan, it is challenging to determine the contribution of exa-cel, which was administered ≥48 hours after completion of conditioning chemotherapy. Please see Table 4:

Table 4. All-Grade and Grade 3-4 Non-Laboratory AEs Reported in ≥10% Exa-cel Recipients by SOC, and Preferred Term, Day 1 to Month 24, N=44 Evaluable Subjects, Studies 121 and 131

System Organ Class **Preferred Term**	**(N=44)** **n (%)**	**Grade 3-4** **n (%)**
Blood and lymphatic system disorders		
Febrile neutropenia	24 (55)	21 (48)
Cardiac disorders		
Tachycardia †	9 (20)	1 (2)
Eye disorders		
Vision blurred	6 (14)	1 (2)
Gastrointestinal disorders		
Mucositis ‡, §	43 (98)	38 (86)
Nausea	31 (70)	4 (9)
Abdominal pain ¶	27 (61)	5 (11)
Vomiting	25 (57)	2 (5)
Constipation	20 (45)	4 (9)
Diarrhea	17 (39)	1 (2)
Gastritis	11 (25)	0
Gastroesophageal reflux disease	8 (18)	0
Dyspepsia	5 (11)	0
Hematochezia	5 (11)	0
General disorders and administration site conditions		
Pyrexia	18 (41)	0
Fatigue	16 (36)	2 (5)
Edema peripheral	12 (27)	0
Pain	11 (25)	3 (7)
Drug withdrawal syndrome	9 (20)	0
Hepatobiliary disorders		
Cholelithiasis	8 (18)	5 (11)
Infections and infestations		
Oral candidiasis	9 (20)	0
Upper respiratory tract infection #	9 (20)	0
Pneumonia	5 (11)	4 (9)
Viral infection ‡, Þ	5 (11)	0

System Organ Class Preferred Term	(N=44) n (%)	Grade 3-4 n (%)
Injury, poisoning and procedural complications		
Procedural pain	9 (20)	1 (2)
Infusion-related reactions [‡, β]	6 (14)	
Investigations		
Weight decreased	8 (18)	3 (7)
Metabolism and nutrition disorders		
Decreased appetite	21 (48)	18 (41)
Musculoskeletal and connective tissue disorders		
Musculoskeletal pain [‡, á]	29 (66)	5 (14)
Arthralgia	19 (43)	3 (7)
Nervous system disorders		
Headache	22 (50)	4 (9)
Dizziness	10 (23)	1 (2)
Paresthesia	5 (11)	0
Psychiatric disorders		
Anxiety	9 (20)	1 (2)
Insomnia	7 (16)	0
Renal and urinary disorders		
Dysuria	7 (16)	0
Respiratory, thoracic and mediastinal disorders		
Oropharyngeal pain [‡, ė]	11 (25)	4 (9)
Epistaxis	9 (20)	2 (5)
Cough	7 (16)	0
Skin and subcutaneous tissue disorders		
Pruritus	22 (50)	5 (11)
Pigmentation disorder [ð]	17 (39)	0
Skin exfoliation	10 (23)	0
Rash [ø]	8 (18)	0
Alopecia	7 (16)	0
Dry skin	6 (14)	0
Vascular disorders		
Hypertension	7 (16)	2 (5)
Hot flush	5 (11)	0

Source: Reviewer derived from ADAE dataset from Day 90 Safety Update
[†] Tachycardia includes sinus tachycardia and tachycardia.
[‡] Encompasses preferred terms that belong to other system organ class.
[§] Mucositis includes anal inflammation, mucosal inflammation, pharyngeal inflammation, and stomatitis.
[¶] Abdominal pain includes abdominal discomfort, abdominal pain, abdominal pain lower, abdominal pain upper, and abdominal tenderness.
[#] Upper respiratory tract infection includes upper respiratory tract infection and viral upper respiratory tract infection.
[Þ] Viral infection includes adenovirus infection, influenza, parvovirus B19 test positive, viral infection, and viral test positive.
[β] Infusion related reactions includes terms on Day 1 of CASGEVY infusion that were consistent with common infusion-related signs and symptoms: abdominal pain in 3 (7%) patients; and infusion related reaction, nausea, non-cardiac chest pain, pruritus, sinus tachycardia and vomiting in 1 (2%) patient each.
[á] Musculoskeletal pain includes back pain, bone pain, chest pain, costochondritis, musculoskeletal chest pain, myalgia, neck pain, non-cardiac chest pain, pain in extremity, and tendon pain.
[ė] Oropharyngeal pain includes oral pain, oropharyngeal pain, and pain in jaw.
[ð] Pigmentation disorder includes nail pigmentation, skin hyperpigmentation, and skin hypopigmentation.
[ø] Rash includes dermatitis, rash, rash macular, rash maculo-papular, rash papular, and urticaria.
Abbreviations: SOC, body system organ class, ADAE, Adverse Event Analysis Data Set; AE, adverse event; exa-cel, exagamglogene autotemcel; n, number of subjects with the specified characteristic; N, number of subjects in the specified group, or the total sample.

6.1.12.3 Deaths

The one subject who died was determined by the investigator to not be related to study drug, and as a result of COVID-19 infection and busulfan. The subject was 33 years of age and had a medical history of pulmonary emboli treated with chronic anticoagulation, SCD-associated episodes of acute chest syndrome, and tobacco smoking with intermittent dyspnea and hypoxia. The subject had an unremarkable mobilization, apheresis, and myeloablative conditioning course prior to receiving exa-cel. She achieved neutrophil engraftment on Day 23 and platelet engraftment on Day 36. She was discharged from the hospital after exa-cel infusion on Day 30. Beginning on Day 71, the subject developed symptoms of cough and rhinorrhea as an outpatient and COVID-19 test (rapid antigen) was positive. A chest X-ray showed bilateral pneumonia with patchy infiltrates and perihilar opacities. The AE of COVID-19 was treated with remdesivir and sotrovimab and resolved on Day 92. On Day 112, the subject was hospitalized for SAEs of pneumonia and hypoxia, which progressed to respiratory failure.

Pathology from a transbronchial lung biopsy showed acute lung injury with features of organizing diffuse alveolar damage and type 2 pneumocytes, suggestive of drug-induced (busulfan) lung injury. The subject had a protracted intensive care unit course with intubation and ECMO support. She had a deteriorating course marked by septic shock, right ventricular failure, and bowel ischemia. Therefore, ECMO support was discontinued according to the subject's wishes, and she died immediately after that. Lung injury and serious infections, including that with a fatal outcome, are known risks of busulfan treatment.

Reviewer Comment:
This subject had an unremarkable initial post exa-cel course before hospital discharge after neutrophil engraftment but had a complicated medical history including pulmonary disorder and risk factors. The reviewer suspects that COVID-19 pneumonia, complicated by underlying cardiopulmonary disease and a component of drug-induced lung injury based on transbronchial bung biopsy, led to the multifactorial fatal respiratory failure. The death is unlikely to have been related to exa-cel.

6.1.12.4 Nonfatal Serious Adverse Events

Following administration of exa-cel, 16 (38.1%) subjects had at least 1 SAE. The most common SAEs included cholelithiasis and pneumonia, which each occurred in 4 (9.1%) subjects. Overall, most SAEs had an onset within the first 6 months after exa-cel infusion.

Table 5. Serious Adverse Events Occurring in ≥2 Subjects by Preferred Term, Day 1 to Month 24, N=44 Evaluable Subjects, Studies 121 and 131

Preferred Term	n (%)
Cholelithiasis	4 (9.1)
Pneumonia	4 (9.1)
Abdominal pain	3 (6.8)
Constipation	3 (6.8)
Pyrexia	3 (6.8)
Sickle cell anemia with crisis	3 (6.8)
Abdominal pain upper	2 (4.5)
Non-cardiac chest pain	2 (4.5)
Oropharyngeal pain	2 (4.5)
Pain	2 (4.5)
Sepsis	2 (4.5)

Source: Reviewer derived from ADAE dataset from Day 90 Safety Update
Abbreviations: n, number of subjects with the specified characteristic; N, number of subjects in the specified group, or the total sample.

Reviewer Comment:
The observed types and rates of AEs and SAEs reported during the period between conditioning and before neutrophil engraftment are reasonably expected from busulfan conditioning. All 44 (100.0%) subjects had ≥1 AE after exa-cel infusion, and Grade 3 or 4 AEs occurred in 40 (95.2%) subjects. While neutropenia was common, severe grade infections were not. There were 2 (4.5%) AEs of sepsis.

6.1.12.5 Adverse Events of Special Interest

Infection

Infection AEs occurred in 29 (65.9%) subjects. Most common AEs of infection (occurring in ≥10% of subjects) were COVID-19 (11 [25.0%]), oral candidiasis (9 [20.5%]), upper respiratory tract infection (7 [15.9%]), and pneumonia (5 [11.4%]). Grade 3 or 4 infection AEs occurred in 10 (22.7%) subjects and infection SAEs occurred in 9 (20.5%) subjects. SAEs of pneumonia (4 [9.1%]) and sepsis (2 [4.5%]) were the only SAEs that occurred 2 or more subjects. Overall, the incidence of infection was consistent with that observed after HSCT.

Bleeding Events

Bleeding AEs occurred in 21 (47.7%) subjects. The majority of bleeding AEs were Grade 1 or 2 in severity. Grade 3 or 4 bleeding AEs occurred in 3 (6.8%) subjects. The most common bleeding AEs (occurring ≥10% subjects) after exa-cel infusion were epistaxis (9 [20.5%]) and hematochezia (5 [11.4%]). The median duration of bleeding AEs was 3.0 (range: 1 to 126) days. Bleeding for 126 days occurred in the subject who eventually died, while she was anticoagulated and was on ECMO. Eleven (25%) subjects had at least 1 bleeding AE that was considered related or possibly related to busulfan. None of the bleeding AEs were considered related or possibly related to exa-cel by the Applicant.

One subject (b) (6) experienced Grade 2 epistaxis that continued for 70 days until Day 84, with a platelet count of only 14/µL on Day 83. This subject had not reached platelet engraftment as of the date of data lock. At the last measurement on Day 114, the platelet count was still only 38/µL.

Additional data from the 90-day safety update showed that the subject's platelets rose and reached platelet engraftment (PE) by Day 126; the last measured platelet count was 102/µL on Day 183.

Reviewer Comment:
The incidence of complications of myelosuppressive bone marrow conditioning, such as febrile neutropenia, was as expected in the conduct of autologous marrow transplant and was likely related to busulfan. The incidence and grade of infection-related AEs are also likely as expected with an autologous transplant. Considering that platelet engraftment was found to be somewhat delayed compared to allo-HSCT, bleeding events were an AE of special interest. Most bleeding were Grades 1 and 2 in severity and were busulfan-related.

6.1.12.6 Clinical Test Results

Laboratory Abnormalities

Shift table analysis was performed to evaluate the impact of exa-cel and myeloablation on a variety of laboratory parameters. The most common Grade 3 or higher laboratory abnormalities included: neutropenia, thrombocytopenia, leukopenia, anemia, and lymphopenia. Platelet and neutrophil engraftment dynamics are discussed separately below. See the Table 6 for detailed listing of all laboratory abnormalities.

Table 6. All Grade and Grade 3 or 4 Laboratory Abnormalities in ≥10% Subjects Treated From Day 1 to Month 24 After Exa-cel Infusion, Studies 121 and 131

Laboratory Abnormality	All Grade Laboratory Abnormalities	% All Grade Laboratory Abnormalities	Grade 3-4 Laboratory Abnormalities	% Grade 3-4 Laboratory Abnormalities
Neutrophils decreased	44	100%	44	100%
Platelets decreased	44	100%	44	100%
Leukocytes decreased	44	100%	43	98%
Hemoglobin decreased	44	100%	37	84%
Lymphocytes decreased	40	91%	22	50%
CD4+ cells decreased	32	73%	10	23%
aPTT increased	35	80%	7	16%
ALT increased	36	82%	5	11%

Source: Calculated by reviewer from ADLB dataset
Abbreviation: ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time

Reviewer Comment:
The laboratory abnormalities are more detailed in the laboratory (ADLAB) dataset as compared to the adverse event (ADAE) dataset. Therefore, the label will

include a separate table for laboratory abnormalities that are derived from the ADLB dataset.
Severe pancytopenia was universally reported. This finding is expected following full myeloablative doses of busulfan that is required with exa-cel therapy. Most subjects required transfusion support with RBCs and platelets. G-CSF was not permitted unless neutrophil engraftment did not occur by Day 21.

Neutrophil Engraftment

Subjects treated with exa-cel remained in the hospital until neutrophil engraftment (NE). This was defined as the first day of 3 consecutive measurements of absolute neutrophil count ≥500/μL on 3 different days, without use of the unmodified CD34+ cells after reaching the nadir, defined as absolute neutrophil count <500/μL. The use of granulocyte colony stimulating factor (GCSF) was permitted if NE did not occur by Day 21. GCSF was administered to 19 subjects, with the latest use occurring on Day 43.

The time to NE was similar in the younger age subpopulation compared to the adults, and the ranges overlapped. This is shown in Table 7.

Table 7. Time to Neutrophil Engraftment by Age, Studies 121 and 131

Parameter	Age 12 to <18 N=12	Age ≥18 N=31	Total N=44
Time to NE median (range)	28 (24-40)	25.5 (15-38)	27 (15-40)
Time to NE mean (SD)	29.8 (4.6)	25.2 (6.2)	26.5 (6.1)

Source: Reviewer derived from ADSL dataset; 90-day safety follow-up submission
Abbreviations: N, number of subjects in group; NE, neutrophil engraftment; SD, standard deviation

Platelet Engraftment

The Study 121 protocol defined platelet engraftment as the first day of 3 consecutive measurements of unsupported (no platelet transfusions in last 7 days) platelets ≥50,000/μL on 3 different days after exa-cel transfusion, after reaching the nadir, defined as platelet <50,000/μL, or the first platelet transfusion, whichever was earlier. For subjects who were discharged before reaching platelet engraftment, platelet engraftment was defined as the seventh day after the last platelet transfusion, if there were 3 subsequent and consecutive unsupported measurements of unsupported platelet ≥50,000/μL on 3 different days. The last platelet transfusion referred to the last platelet transfusion preceding these 3 measurements.

Recipients of exa-cel reached PE after a median of 35 days (range 23 to 126). The results are shown in Table 8.

Reviewer Comment
Subjects experienced a delay in platelet engraftment compared with the published outcomes of patients with SCD undergoing allo-HSCT. This does not appear to have been associated with greater risk of hemorrhagic complications

and did not lead to thrombopoietin agonist use. The etiology of this delay is unknown.

Time to PE was analyzed by age subgroup (adolescents compared to adults). Utilizing the data from the 90-day safety follow-up, the time required to reach the definition of platelet engraftment was analyzed by age 12 to <18 years versus ≥18 years. Among the N=12 adolescents <18 years old, the median time to PE was 44.5 days (23 to 81) compared with 32 days (23 to 126) in adults. Mean time to PE in adolescents was numerically slower than in adults. Please see Table 8.

Table 8. Time to Platelet Engraftment by Age, Studies 121 and 131

Parameter	Age 12 to <18 N=12	Age ≥18 N=31	Total N=44
Time to PE median (range)	44.5 (23-81)	32 (23-126)	35 (23-126)
Time to PE mean (SD)	46.2 (17.2)	42 (24)	43.2 (22.2)

Source: Reviewer derived from ADSL dataset; 90 safety follow-up submission
Abbreviations: N, number of subjects in group; PE, platelet engraftment; SD, standard deviation

Reviewer Comment:
The reason for the possible latency to PE by adolescents compared with adults is unclear. The observation may indicate a trend and thus increases the importance of close observation of time to PE and bleeding AEs especially among children <12 years old in any future pediatric trials.

Bone Marrow Pathology Review

Considering that prolonged cytopenia may be a signal of marrow pathology, the risks of off-target gene editing are unknown, and that busulfan conditioning can be associated with a potential risk of hematologic malignancy, the review team reviewed the pathologic results of bone marrow biopsy and aspirates, focusing especially on those subjects with the slowest platelet engraftment. We selected for review those 11 subjects with time to PE ≥50 days and sent an IR requesting pathology reports. Bone marrow biopsies were performed on most subjects at baseline and at 6, 12, and 24-months post exa-cel for the purpose of assessing allele editing and not to evaluate cytopenias or exclude other pathology; thus, some of the subjects did not have formal pathology reports for the team's review. Not all subjects had sufficient follow-up to have 12- or 24-month assessments. Based on review of these available bone marrow pathology reports, there was no malignancy, increased blasts, or dysplasia observed. One subject had pericentric inversion of the ninth chromosome, but inv(9)(p12q13) can be a benign variant and occurs in ≤2% of the general population.

Reviewer Comment: Limited review of available bone marrow pathology reports did not reveal concerning findings within exa-cel recipients with slower platelet engraftment.

Secondary Erythrocytosis

The oxygen dissociation curve of HbF is shifted to the left relative to adult hemoglobin, which means that HbF binds to oxygen more avidly, and unloads it less easily at tissue level (Wardle et al. 1998). Increased expression of HbF among exa-cel recipients is predicted to theoretically induce relative tissue hypoxemia, predisposing to secondary erythrocytosis.

The review team screened hemoglobin levels obtained after hematopoietic reconstitution in exa-cel recipients and found three male subjects who experienced the greatest increase in total hemoglobin levels, with peak Hb levels between 16.5 to 17.9g/dL, with upgoing trends in three of the four cases. The NHANES and Scripps-Kaiser databases suggest a mean hemoglobin level for Black men aged 30-39 in g/dL (SD) of 14.61(0.96), and 14.76 (0.76), respectively[2]; and thus, an upper limit of normal (ULN) value of 16.28-16.53g/dL, respectively. As their Hb persistently exceeded the ULN, these subjects have erythrocytosis. An IR was sent, to clarify if a reason for the erythrocytosis was known, asking about medical history of the subjects.

Per IR response, none of these subjects smoked. Subject (b) (6) (peak Hb 18.1g/dL at local lab, 17.9g/dL at central lab) was a 34-year-old male with history of myocardial infarction. Following the peak Hb level, he underwent two phlebotomies for hemochromatosis. Subject (b) (6) had intermittent HTN, and subject (b) (6) had cardiomegaly, dilated left atrium and mildly dilated left ventricle, mild mitral valve and tricuspid regurgitation, and asthma.

One female subject (b) (6) with erythrocytosis was also identified in study 121, with Hb levels as high as 15.7g/dL.

It is difficult to draw conclusions from trends in the available data; erythropoietin measurement was incorporated into the study 121 protocol late, consequently, many subjects lack a baseline, and other values early in their course.

Reviewer comment: SCD is almost universally associated with anemia, therefore the development of erythrocytosis following exa-cel is notable. Living with SCD for decades, patients may accumulate cardiovascular damage with increased risk of stroke and myocardial infarction, making them less tolerant of sudden increases in viscosity from rheostatic changes ensuing after exa-cel treatment and Hb levels approaching or exceeding ULN. Although secondary erythrocytosis usually does not carry risk of thrombosis seen with polycythemia vera, it is unknown if production of therapeutically augmented levels of HbF seen

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2 Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? Blood. 2006 Mar 1;107(5):1747-50. doi: 10.1182/blood-2005-07-

among exa-cel recipients and consequent secondary erythrocytosis further exacerbate these risks in those who accrued SCD-related cardiovascular changes before exa-cel. The USPI will report on these hemoglobin levels, and to further evaluate this safety signal the PMR#1 safety study will report Hb levels over a 15 year period.

Infusion-Related Reactions

Overall, no clinically significant infusion-related reactions were observed and the exa-cel infusion was well tolerated. All potential infusion-related AEs assessed on the day of exa-cel infusion were non-serious and were mild or moderate in severity. There were no anaphylactic-type events following exa-cel infusion at any time during the study.

Since infusion-related reactions may be reported as clinical events (e.g., chills), to identify any potential infusion-related events, all AEs that occurred on Study Day 1 were reviewed and evaluated by the sponsor to determine if any events were consistent with common infusion-related signs and symptoms or hypersensitivity-type reactions. This analysis revealed a total of 6 subjects who had a potential infusion-related AE. The only AE occurring in >5% of subjects was abdominal pain. All potential infusion-related AEs were mild or moderate in severity and none were serious. The majority of events were consistent with known side effects of busulfan, which was administered within the week before exa-cel infusion.

Veno-Occlusive Liver Disease (VOLD)

A single subject (2.3%) had a non-serious, Grade 3 AE of VOLD that resolved within 12 days. This subject was treated with prophylactic ursodeoxycholic acid starting from the time of busulfan conditioning and continuing after exa-cel infusion and was subsequently treated with defibrotide for the AE. The event was considered related to busulfan. No other AEs of VOLD have occurred on study. The protocol suggested prophylaxis for veno-occlusive liver disease using defibrotide per investigator's discretion in accordance with standard of care.

Reviewer Comment: The overall incidence and pattern of VOLD events is consistent with the literature for subjects with SCD undergoing busulfan-based myeloablative conditioning and HSCT. Recommendation to consider prophylaxis will be communicated in USPI.

Safety in Special Populations (Adolescents)

The observed safety profile was generally similar between subjects <18 and ≥18 of age. AEs were largely consistent with myeloablative busulfan conditioning, HSCT, and underlying disease. As discussed above, time to platelet engraftment was numerically longer among adolescents than adults, although the clinical implications of this were not apparent. Please see Table 9 below:

Table 9. Treatment-Emergent Adverse Events, by Age Group, Study 121

Parameter	≥12 and <18 years n (%)	≥18 and ≤35 years n (%)
Evaluable subjects, N	12	32
Subjects with any AE	12 (200)	32 (100)
Subjects with Grade 3 or 4 AEs	5 (41.7)	15 (46.9)
Subjects with SAEs	5 (41.7)	15 (12.5)
Subjects with AEs leading to death	0	2 (3.1)

Source: Reviewer calculations from ADAE dataset.
Abbreviation: AE, adverse event; N, number of subjects in treatment group; n, number of subjects in subgroup; SAE, serious adverse event

Sex and Race

No clinically relevant differences attributable to exa-cel were identified based on sex, race, or SCD genotype, although interpretation is limited due to preponderance of subjects with βS/βS genotype and Black or African American race.

Supportive Safety Data

The Applicant submitted to this BLA, the safety data and safety summary from their ongoing study of exa-cel for transfusion dependent thalassemia (TDT) which was also submitted to another BLA under FDA review. While TDT is a different indication, the safety data can be supportive across both indications, since the mechanism of action and manufacturing process (including the same Cas9 enzyme and gRNA) of exa-cel are identical. The study in TDT (Study 111) enrolled subjects aged ≥12 to 35 years and required the same myeloablative conditioning. The safety profile for TDT was similar to that observed in the SCD population.

6.1.12.7 Dropouts and/or Discontinuations

One subject discontinued after exa-cel infusion, due to death. This was discussed above in Section 6.1.12.3.

6.1.12.8 Additional Safety Evaluations

Dose Dependency for Adverse Events

A single dose was administered to all recipients, with a minimal threshold of 3 x 10^6 CD34+ cells/kg. Dose range or dependency was not studied.

Time Dependency for Adverse Events

Most AEs were reported early after exa-cel administration, as expected, considering that full myeloablative conditioning shortly preceded exa-cel. The majority (≥70%) of AEs, SAEs, and Grade 3 or above AEs, occurred in the first 6 months after exa-cel infusion. The number and time-adjusted rate (events/patient-months) of AEs, Grade 3 or above AEs, and SAEs was highest within the first 6 months following myeloablative conditioning with busulfan and

exa-cel infusion, as compared to all the following 6 months intervals (i.e., 6 to <12, 12 to <18, and 18 to 24 months). Data from IA2, with a data lock date of February 10, 2023, are shown in Table 10:

Table 10. Adverse Events and Time-Adjusted Rates of Adverse Events, by Time of SCD Onset After Exa-cel Infusion, Study 121, FAS (from IA2 for Initial BLA)

Category	Exa-cel Infusion to <6 Months	6 Months to <12 Months	12 Months to <18 Months	≥18 Months
Evaluable subjects, N1	42	35	30	17
Exposure (patient-months)	231.2	192.9	140.8	91.5
Subjects with any AEs, n (%)	42 (100.0)	24 (68.6)	18 (60.0)	12 (70.6)
Total Number of AEs	1470	188	96	48
Subjects with any Grade 3/4 AEs, n (%)	40 (95.2)	8 (22.9)	5 (16.7)	3 (17.6)
Total Number of Grade 3/4 AEs	345	30	9	8
Subjects with any SAEs, n (%)	14 (33.3)	6 (17.1)	2 (6.7)	3 (17.6)
Total Number of SAEs	44	11	2	4
Time-Adjusted AE Rates (Events/patient-months)				
All AEs	6.358	0.975	0.682	0.524
AEs related to exa-cel	0.095	0.010	0.007	0
Grade 3/4 AEs	1.492	0.156	0.064	0.087
SAEs	0.190	0.057	0.014	0.044
SAEs related to exa-cel	0	0	0	0

Sources: Study 121/Table 14.3.1.1.3 (data cutoff date of 10 February 2023)
AEs: adverse event; exa-cel: exagamglogene autotemcel; FAS: Full Analysis Set; n: size of subsample; IA: interim analysis; SAE: serious adverse event; SAS: Safety Analysis Set; SCD: sickle cell disease
Notes: MedDRA version 25.1. Evaluable subjects, N1: The number of subjects in the SAS who were on or after the start date of each study interval (i.e., FAS). Percentages were calculated as n/N1*100. When summarizing number of events for each study interval, a subject with multiple events within a category and study interval was counted multiple times in that category and study interval. When summarizing number and percentage of subjects for each study interval, a subject with multiple events within a category and study interval was counted only once in that category and study interval. An AE with relationship missing to exa-cel was counted as related to exa-cel in this table.
AE onset Month = (AE start date – exa-cel infusion date + 1)/30. Study Day 1 is the day of exa-cel infusion. One month is 30 days. Exposure (patient-months) within each interval = Sum of the follow-up duration (months) within each interval from subjects who are in each interval. The follow-up duration (months) within each interval = (Data cutoff date or end date of each interval whichever is earlier - start date of each interval + 1)/30. The follow-up duration within each interval is only calculated for subjects who are in each interval. Events/patient-months within each interval = Total number of events within each interval / Exposure (patient-months) within each interval.

Source: Modified from Exa-cel SCD Clinical Overview Addendum: Efficacy and Safety Update 14 June 2023, Page 58.

Human Carcinogenicity

Although no malignancies have been reported in any subjects treated on study, CRISPR genome editing may be complicated by potential off-target edits, which theoretically may result in complications, including malignancy. The latency from treatment to such an event is unknown. The Applicant has performed in silico and cell-based screening of their gRNA and CRISPR/Cas9, in order to minimize these risks. Please see CMC and Bioinformatics review memoranda for details about off-target editing risks.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

There were no reports of overdose of exa-cel and, considering the nature of this product, this would be highly unlikely. . Exa-cel has no drug abuse potential, being an autologous HSCP product, and is not expected to lead to withdrawal or rebound.

Immunogenicity (Safety)

Exa-cel is an autologous HSPC product wherein the target of the gene modification is expected not to insert a new gene, but rather to downregulate an

existing gene (BCL11A), with the goal of increasing expression of gamma globin and HbF. Gamma globin and HbF exist in all humans, thus, unless CRISPR/Cas9 were to cause off target edits and produce neo peptides, immunogenicity is unexpected. Please see the Pharmacology/Toxicology review memo for details of preclinical work to determine risks of immunogenicity with this product, and Bioinformatics review memo for details of off-target editing.

Person-to-Person Transmission, Shedding

The autologous HSPCs are unexpected to lead to person-to-person transmission or shedding.

6.1.12.9 Safety Conclusions

Overall, the safety profile demonstrated with exa-cel use in the population studied in Study 121 is comparable to that expected following autologous HCT, though the time to platelet engraftment is prolonged versus allo-HSCT. There exists theoretic potential for off-target genome editing, which was the focus of the AC hearing and will be addressed with a PMR study.

6.1.13 Study Summary and Conclusions

Study 121 demonstrated evidence of efficacy, while the overall safety profile is as expected with an autologous HSCT.

6.2 Study 131

Study 131, titled “Long-term Follow-up Study of Subjects With β-thalassemia or Sickle Cell Disease Treated with Autologous CRISPR-Cas9 Modified Hematopoietic Stem Cells” is an ongoing long-term roll over, follow-up study. Its main objectives are to evaluate safety and efficacy for up to 15 years following exa-cel infusion in subjects with TDT and SCD.

The inclusion criteria encouraged enrollment of any consenting subjects dosed with exa-cel in the TDT and SCD studies, as well as anyone who discontinued after being dosed. There were no exclusion criteria.

Study 131 evaluated the subjects from Study 121 for the same efficacy and safety endpoints as in Study 121, but every 3 months until end of Year 3, every 6 months for Year 4 and Year 5, and then annually thereafter until up to Year 15.

Endpoints collected for efficacy included: severe VOC events, inpatient hospitalizations for severe VOCs, hemolysis markers (reticulocytes/erythrocytes, lactate dehydrogenase, haptoglobin, and total and indirect bilirubin), and SCD-specific and pain scale PROs.

Safety endpoints included: new malignancies, new or worsening hematologic disorders, all-cause mortality, all serious adverse events (SAEs) occurring up to 5 years after exa-cel infusion, and all AEs and SAEs related to exa-cel. In addition, AEs of SCD-related complications were evaluated for subjects with

severe SCD only. For safety, subjects were evaluated with abbreviated physical examinations, laboratory and imaging assessments, and AEs. Descriptive analysis of safety was performed; no statistical testing was performed.

The review team analyzed efficacy and safety based on data from study 121 and 131, as discussed above. To date, the only AE reported following Month 24, among the N=8 subjects with SCD who graduated from Study 121 into Study 131, was a case of noroviral gastroenteritis, which was self-resolving after 4 days, on Day 802. This was considered unrelated to study treatment.

7. INTEGRATED OVERVIEW OF EFFICACY

An integrated analysis of efficacy was not conducted since there was only one pivotal study.

8. INTEGRATED OVERVIEW OF SAFETY

An integrated analysis of safety was not conducted since there was only one pivotal study.

9. ADDITIONAL CLINICAL ISSUES

9.1 Special Populations

9.1.1 Human Reproduction and Pregnancy Data

Exa-cel was not studied in pregnant women and no data are available. However, subjects were required to undergo myeloablative conditioning with busulfan, which has well known reproductive and pregnancy toxicity. All subjects of childbearing potential should be informed of this risk and advised regarding fertility preservation.

9.1.2 Use During Lactation

No data are available.

9.1.3 Pediatric Use and PREA Considerations

Exa-cel has orphan designation and is Pediatric Research Equity Act (PREA) exempt. Studies to date have evaluated use of exa-cel in subjects ≥12 years of age.

9.1.4 Immunocompromised Patients

Exa-cel has not been evaluated in immunocompromised subjects.

9.1.5 Geriatric Use

Exa-cel has not been evaluated in geriatric subjects, nor those over age 35.

10. CONCLUSIONS

Efficacy

Efficacy of exa-cel is based on prevention of sVOCs over a period of at least 12 months, demonstrated in a multicenter, open-label, single-arm clinical trial with adolescents and adults who had recurrent sVOCs. A total of 44 subjects were infused and 29/31 (93.5%) efficacy evaluable subjects achieved VF12. All 30 (100%) of evaluable subjects achieved the key secondary efficacy endpoint (HF12). The efficacy outcomes were generally consistent across subgroups with respect to age, sex, and genotype. The basis of FDA's conclusion of substantial evidence of effectiveness is the magnitude of benefit driven primarily by the rate of VF12.

In summary, Study 121 represents an adequate and well-controlled trial that provides substantial evidence of effectiveness of exa-cel for the treatment of SCD in patients 12 years and older with recurrent VOCs. The results support a regular approval for exa-cel.

Safety

The safety profile of exa-cel therapy entails the rigors of myeloablation with frequent cytopenias and gastrointestinal symptoms, which resolved. Delayed platelet engraftment was observed, although without clinical sequalae. Due to off-target editing risks inherent to CRISPR/Cas9 gene therapy, a PMR safety study will be required. In summary, Study 121 represents an adequate and well-controlled study that provided substantial evidence of effectiveness and safety.

11. RISK-BENEFIT CONSIDERATIONS AND RECOMMENDATIONS

11.1 Risk-Benefit Considerations

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Sickle cell disease (SCD) is a hereditary hemoglobinopathy characterized by production of HbS, which deforms red cells in a deoxygenated state, leading to hemolysis and vaso-occlusive crises. • It is the most common hemoglobinopathy in the U.S., affecting about 80,000 people.	• SCD impacts the U.S. population and is associated with substantial morbidity and mortality. • SCD leads to shortened survival due to cumulative organ damage, though fulminant events such as acute chest syndrome or stroke, may be immediately fatal. Adults with SCD have a life expectancy of approximately 45 years of age.
Unmet Medical Need	• The sole FDA-approved drug to treat SCD for over 20 years, hydroxyurea, has modest effect, largely mediated by causing increased HbF expression. Over the last decade, additional drugs have been approved including crizanlizumab, voxelotor, and L glutamine. These drugs are not curative and offer modest benefit. • Hematopoietic stem cell transplantation from a matched donor is a potentially curative treatment option for pediatric patients, but this modality is limited by lack of appropriate donors and potential risks of stem cell transplantation, including graft versus host disease.	• Only a small minority of SCD patients have an appropriate HSPC transplant donor. Consequently, an unmet medical need for therapeutic options exists because of insufficient suitable donors.
Clinical Benefit	• Exa-cel treatment demonstrated clinically meaningful reduction in VOCs, achieving the primary efficacy endpoint of absence of VOCs for at least 12 months following infusion of exa-cel, in the vast majority of subjects, along with complete absence of hospitalizations for VOC (VH12) for at least 12 months following exa-cel, and supported by pharmacodynamics, revealing a robust underlying expression of HbF median of ~40% staring after month 3 post exa-cel. • These results were durable and robust as demonstrated by consistency across all subgroups.	• Exa-cel treatment resulted in absence of severe VOCs in most subjects for at least 12 months. • All subjects achieved VH12, requiring no hospitalization for VOCs for at least a 12-month period. • Subjects expressed HbF of 40% persistently starting from about 3 months post exa-cel.
Risk	• The most common adverse reactions (incidence ≥25%) were mucositis, nausea, musculoskeletal pain, abdominal pain, vomiting, febrile neutropenia, headache, pruritus, decreased appetite, constipation, arthralgia, pyrexia, diarrhea, pigmentation disorder, fatigue, edema peripheral, gastritis, oropharyngeal pain, and pain. • Serious adverse reactions were observed in 45% of subjects with SCD. The most common serious adverse reactions (≥2 patients) were cholelithiasis, pneumonia, abdominal pain, constipation, pyrexia, abdominal pain upper, non-cardiac chest pain, oropharyngeal pain, pain, and sepsis. • However, risks due to off-target editing potential from CRISPR/Cas9 involved in manufacture of exa-cel are unknown.	• The overall risk for the proposed population is acceptable. • Long-term safety will be an important consideration and will be part of a post-marketing requirement/ commitment considering the first in human gene edit CRISPR Cas9 technology used. • To minimize risks, labeling will include warnings and precautions for delayed platelet engraftment and prolonged thrombocytopenia. • There was no discontinuation due to adverse event.

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
Risk Management	• Warnings and instructions in the package insert, the PMR studies, and the pharmacovigilance plan are adequate to manage the risks.	• The PMR studies and routine measures, such as the package insert, and pharmacovigilance plan, would b adequate to manage the risks

11.2 Risk-Benefit Summary and Assessment

SCD is a severe disease with recurrent sVOCs, organ damage, and shortened lifespan. The only potential curative treatment option is allo-HSCT, but most patients lack appropriate HSC donors.

Among 44 subjects treated with exa-cel, 31 are VF12 evaluable and of these, 29 (93.5%) have achieved VF12, reflective of clinical benefit. This outcome supports a statistically significant and very persuasive clinical benefit of exa-cel on irreversible morbidity i.e., vaso occlusive crises in SCD. The primary endpoint is further supported by secondary endpoints and pharmacodynamic parameters.

Submitted data provide evidence of benefit with exa-cel treatment. The safety profile indicates delayed platelet engraftment in addition to toxicities expected from prerequisite myeloablation. Although there exists a potential for off-target genome editing complications, there are no identified cases of any such complications identified thus far. The benefit-risk of exa-cel for the treatment of SCD with recurrent sVOCs is favorable.

11.3 Discussion of Regulatory Options

The Applicant has provided substantial evidence of effectiveness and safety from one trial for exa-cel treatment of SCD. The provided data have demonstrated evidence of effectiveness of exa-cel, while indicating that safety is largely consistent with the prerequisite myeloablation and delayed platelet engraftment. On this basis, exa-cel will be granted regular approval.

11.4 Recommendations on Regulatory Actions

No gene therapy products are currently approved for SCD treatment. The review team recommends regular approval for exa-cel for the treatment of sickle cell disease in patients 12 years and older with recurrent vaso-occlusive crises.

11.5 Labeling Review and Recommendations

The review team discussed with the Applicant several sections of the label, resulting in the prescribing information published at the time of BLA approval.

11.6 Recommendations on Postmarketing Actions

The Applicant will conduct two PMR safety studies. PMR#1 will be a postmarketing, prospective, multicenter observational study to assess and characterize the risks of secondary malignancies and off-target effects following genome editing occurring after treatment with exagamglogene autotemcel, and to assess the long-term safety of exagamglogene autotemcel. The study will include 250 subjects with SCD who received/will receive exagamglogene autotemcel, and each enrolled subject will be followed for 15 years after product

administration. The study design will include monitoring (at prespecified intervals) with adequate testing strategies (Study Protocol VX22-290-101).

The second study (PMR#2) will be an (i) in silico off-target analysis using publicly available databases/datasets to allow for inclusion of more variants. Specifically, analysis will be performed using all variants with at least 0.5%allele frequency in at least one of the five continental groups (Africa, Europe, East Asia, South Asia, and the Americas).

ii. Confirmatory testing will be performed, as appropriate and feasible, of all the off-target loci nominated from the new in silico analysis from (i) as well as those that were not accounted for in the previous study using appropriate samples harboring variants.

a. Screening will be done for the presence of all previously identified variants (e.g., CPS1) as well as any variants identified in study (i) and (ii) in the patients treated in Studies 121, 111, 141, 151, 161, and 171.

b. For patients with a confirmed variant(s), assessment shall be done for indels and chromosomal changes at each respective locus in appropriate samples.