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FDA Briefing Document

BLA# 125787/0

Drug name: exagamglogene autotemcel

Applicant: Vertex Pharmaceuticals Inc.

Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

10/31/23

Office of Therapeutic Products

DISCLAIMER STATEMENT

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the Advisory Committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Divisions or Offices. We bring benefit-risk of exagamglogene autotemcel for sickle cell disease patients with recurrent vaso-occlusive crises to this Advisory Committee in order to gain the Committee's insights and opinions. The background package may not include all issues relevant to the final regulatory recommendation and instead, is intended to focus on issues identified by the Agency for discussion by the Advisory Committee. The FDA will not issue a final determination on the issues at hand until input from the Advisory Committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the Advisory Committee meeting.

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Glossary

AC	Advisory Committee
AE	adverse event
BCL11A	B-cell lymphoma/leukemia 11A
BLA	Biologics License Application
CD34+	cluster of differentiation 34+
CRISPR/Cas9	clustered, regularly interspaced, short palindromic repeats-associated 9 nucleases
dsODN	double-stranded oligodeoxynucleotides
DSB	double-strand DNA break
EAC	Endpoint Adjudication Committee
exa-cel	exagamglogene autotemcel
FAS	Full Analysis Set
FDA	Food and Drug Administration
GUIDE-seq	Genome-wide Unbiased Identification of DSBs Enabled by Sequencing
gRNA	guide RNA
HbF	fetal hemoglobin
HbS	sickle hemoglobin
HGDP	Human Genome Diversity Project
HF12	freedom from inpatient hospitalization for severe VOCs sustained for at least 12 months
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation
HSPC	hematopoietic stem and progenitor cell
IA2	interim analysis 2
LTFU	long-term follow-up
NGS	next-generation sequencing
PAM	protospacer adjacent motif
RBC	red blood cells
SAE	serious adverse event
SCD	sickle cell disease
SD	standard deviation
sgRNA	single guide RNA
sVOC	severe vaso-occlusive crisis

TDT	transfusion-dependent β-thalassemia
VF12	freedom from severe VOCs for 12 months from infusion of exa-cel
VOC	vaso-occlusive crisis
WGS	whole genome sequencing

1. Executive Summary/Draft Points for Consideration by the Advisory Committee

1.1 Purpose/Objective of the Advisory Committee Meeting

The FDA is convening this Advisory Committee (AC) meeting to discuss the Applicant's analysis of off-target alterations following editing of patient hematopoietic stem and progenitor cells (HSPCs) with clustered, regularly interspaced, short palindromic repeats-associated protein 9 nucleases (CRISPR/Cas9), and whether it provides an adequate safety assessment.

1.2 Context for Issues to Be Discussed at the Advisory Committee Meeting

Sickle cell disease (SCD) is a hemoglobinopathy characterized by the presence of a mutated hemoglobin subunit beta gene, resulting in sickle hemoglobin (HbS) which polymerizes within red blood cells (RBCs).[1] These deformed, sickle shaped RBCs aggregate causing vaso-occlusion, tissue ischemia and organ damage, and hemolysis. The hallmark of SCD clinical manifestation is vaso-occlusive crises (VOCs), which most commonly are severe painful events, but may also present with acute chest syndrome, priapism, or hepatic/splenic sequestration. In the longer term, SCD may lead to life-threatening neurologic, pulmonary, cardiac, and renal complications and a shortened life span (survival). Available treatment for SCD includes hydroxyurea, L-glutamine, voxelotor, crizanlizumab, as well as allogeneic hematopoietic stem cell transplantation (HSCT). Allogeneic HSCT may be curative, but availability is limited to the small minority with appropriate donors, thus SCD treatment constitutes an unmet need.

Exagamglogene autotemcel (hereafter referred to as exa-cel) is a cell-based gene therapy product composed of autologous, cluster of differentiation 34+ (CD34+) cells edited by CRISPR/Cas9 at the erythroid lineage specific enhancer region of B-cell lymphoma/leukemia 11A (*BCL11A*) gene on chromosome 2, which disrupts GATA1 binding and abrogates *BCL11A* expression, thus un-inhibiting γ-globin expression and fetal hemoglobin (HbF) production. Elevated HbF production within RBCs recapitulates the natural scenario of compound heterozygosity of HbS and the hereditary persistence of HbF, which is often marked by expression of ~30% HbF with pan-cellular distribution. In a manner similar to that observed in individuals with SCD and hereditary persistence of fetal hemoglobin this genetic alteration is therefore expected to reverse SCD manifestations.[2]

[1] For the purposes of this AC meeting, SCD is defined as sickle cell anemia, sickle beta-plus thalassemia, and sickle beta-zero thalassemia, but excludes sickle hemoglobin-C disease.

[2] Ngo, DA, B Aygun, I Akinsheye, JS Hankins, I Bhan, HY Luo, MH Steinberg, and DH Chui, 2012, Fetal haemoglobin levels and haematological characteristics of compound heterozygotes for haemoglobin S and deletional hereditary persistence of fetal haemoglobin, Br J Haematol, 156(2):259-264.

The exa-cel drug product is produced by genome editing of patient HSPCs using CRISPR/Cas9 genome editing. CRISPR/Cas9 genome editing has the potential to produce unintended genomic alterations or off-target editing. These changes can occur both at or near the target site or at other off-target regions in the genome with homology to the guide RNA (gRNA) sequence. These unintended alterations can also occur at different frequencies and in different locations in the genome based on sequence variation in the target population. In their evaluation of off-target editing for exa-cel in the target population, the Applicant used both in silico and cell-based assays. However, the limited amount of sequencing data present in the reference database for the in silico analysis may not adequately capture variants in this population. For the cellular off-target analysis, the Applicant used HSPCs from a small number of healthy donors, transfusion-dependent β-thalassemia (TDT), and SCD donors. Additionally, the healthy donor cells may not adequately capture off-target editing in exa-cel due to potential differences in the chromatin landscape in SCD donor cells.[3]

1.3 Brief Description of the Issue for Discussion at the AC

The Applicant used in silico and cellular assays for off-target assessment of exa-cel. In silico analysis methods use gRNA sequence information and user-provided mismatch criterion while scanning the human reference genome to identify potential off-target editing sites based on sequence homology. Since this analysis scans only the reference genome sequence, potential off-target editing sites in the target (SCD) population that may arise due to nucleotide variations present in individual genomes may not have been sufficiently accounted for. To account for genome heterogeneity in the assessment of off-target editing in the target population, the Applicant performed variant-aware homology search and identified 50 additional off-target loci. For this analysis, they used the 1,000 genomes project database that has whole genome sequencing (WGS) data for 2,504 individuals. Of this, sequencing data were from 661 individuals of the target population. In this group, only 61 WGS datasets were collected in the United States and all datasets were from individuals who were located in the southwest United States. The small target sample size (61 WGS datasets) in this database may not be sufficient for the safety assessment as it may not adequately capture variants in this population across the United States. Furthermore, a recent study by Cancellieri et al. (2023)[4] published a tool, CRISPRme, that allows in silico off-target analysis of a gRNA by including user-provided variant information. The authors of this study used variant information from the Human Genome Diversity Project (HGDP) to nominate potential off-target sites for a gRNA that targeted the same gene as the Applicant's drug product in this BLA. The HGDP dataset contains sequencing information for 929 individuals who were from regions that were not represented

[3] Xi, C, J Pang, W Zhi, CS Chang, U Siddaramappa, H Shi, A Horuzsko, BS Pace, and X Zhu, 2023, Nrf2 sensitizes ferroptosis through l-2-hydroxyglutarate-mediated chromatin modifications in sickle cell disease, Blood, 142(4):382-396.

[4] Cancellieri, S, J Zeng, LY Lin, M Tognon, MA Nguyen, J Lin, N Bombieri, SA Maitland, MF Ciuculescu, V Katta, SQ Tsai, M Armant, SA Wolfe, R Giugno, DE Bauer, and L Pinello, 2023, Human genetic diversity alters off-target outcomes of therapeutic gene editing, Nat Genet, 55(1):34-43

in the 1,000 genomes project database and hence is considered more diverse compared to the latter database.[5] The authors identified an off-target site arising in the intronic region of the *CPS1* gene because of a variant rs114518452 present in the African ancestry samples. We note that the variants identified in this study did not overlap with variants reported by the Applicant. This could be due to potential differences in the in-silico analysis algorithms used, or insufficient sampling of variant information within each dataset, leading to disparate findings between the two studies.

The Applicant used HSPCs from a limited number of samples from healthy subjects (n=3), TDT subjects (n=3), and SCD subjects (n=3) to perform cellular off-target analysis. It is unclear whether the analysis using this limited sample size will provide for an adequate understanding of the potential risk of off-target editing. In addition, SCD has been reported to impact stress,[6,7] chromatin,[8] and HSPC function.[9,10] These factors can potentially change the chromatin landscape in SCD donor cells compared to healthy donor cells. Chromatin accessibility is known to impact off-target editing and contribute to cell-specific differences in off-target loci identification.[11,12] Hence, it is not clear if the limited SCD donor cells used for the off-target assessment will adequately inform the potential safety risks of exa-cel.

1.4 Draft Point for Consideration

Please discuss whether the off-target analysis (e.g., in silico and cellular methods) performed by the Applicant was adequate to assess risk in the intended patient population in the United States or if additional studies should be performed to inform the risk of off-target editing in patients who receive exa-cel for treatment of SCD.

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[5] Bergström, A, SA McCarthy, R Hui, MA Almarri, Q Ayub, P Danecek, Y Chen, S Felkel, P Hallast, J Kamm, H Blanché, JF Deleuze, H Cann, S Mallick, D Reich, MS Sandhu, P Skoglund, A Scally, Y Xue, R Durbin, and C Tyler-Smith, 2020, Insights into human genetic variation and population history from 929 diverse genomes, Science, 367(6484).

[6] Hoppe, CC, 2014, Inflammatory mediators of endothelial injury in sickle cell disease, Hematol Oncol Clin North Am, 28(2):265-286.

[7] Hebbel, RP and GM Vercellotti, 2021, Multiple inducers of endothelial NOS (eNOS) dysfunction in sickle cell disease, Am J Hematol, 96(11):1505-1517.

[8] Xi, C, J Pang, W Zhi, CS Chang, U Siddaramappa, H Shi, A Horuzsko, BS Pace, and X Zhu, 2023, Nrf2 sensitizes ferroptosis through l-2-hydroxyglutarate-mediated chromatin modifications in sickle cell disease, Blood, 142(4):382-396.

[9] Leonard, A, A Bonifacino, VM Dominical, M Luo, JJ Haro-Mora, S Demirci, N Uchida, FJ Pierciey, Jr., and JF Tisdale, 2019, Bone marrow characterization in sickle cell disease: inflammation and stress erythropoiesis lead to suboptimal CD34 recovery, Br J Haematol, 186(2):286-299.

[10] Javazon, EH, M Radhi, B Gangadharan, J Perry, and DR Archer, 2012, Hematopoietic stem cell function in a murine model of sickle cell disease, Anemia, 2012:387385.

[11] Kim, D and JS Kim, 2018, DIG-seq: a genome-wide CRISPR off-target profiling method using chromatin DNA, Genome Res, 28(12):1894-1900.

[12] Guo, C, X Ma, F Gao, and Y Guo, 2023, Off-target effects in CRISPR/Cas9 gene editing, Front Bioeng Biotechnol, 11:1143157.

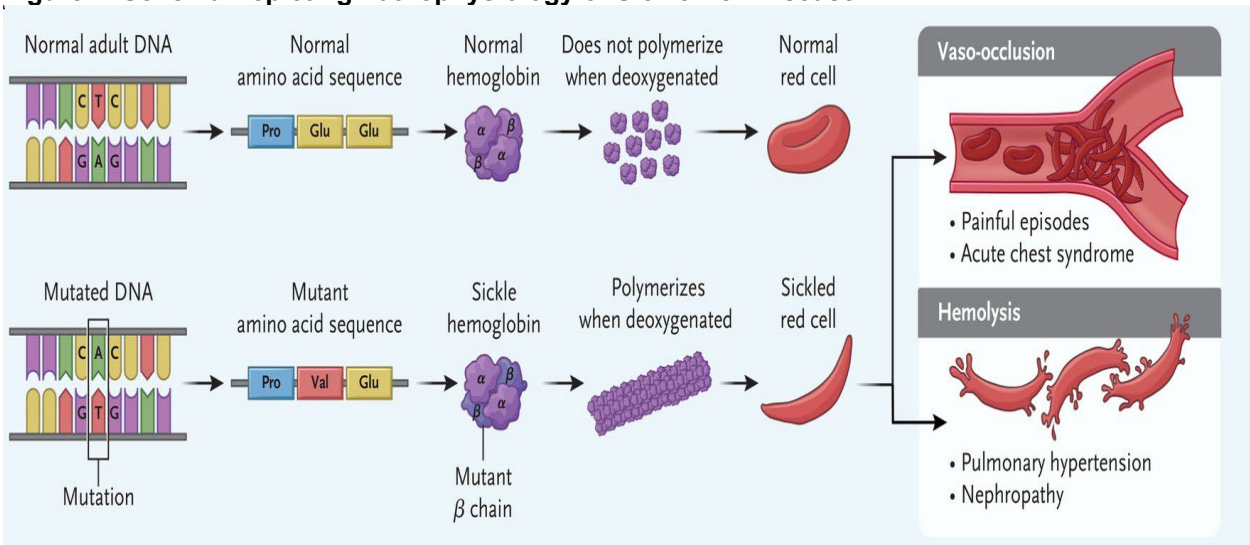
2. Introduction and Background

2.1 Background of the Condition/Standard of Clinical Care

SCD is a group of hemoglobinopathies such as sickle cell anemia, sickle beta-plus thalassemia, and sickle beta-zero thalassemia.[13] SCD largely affects persons mainly of African or Mediterranean ancestry, including an estimated 70,000 U.S. patients.[14,15] Affected patients experience severe painful VOCs and organ damage, involving kidney, cardiopulmonary, and brain. Although a number of pharmaceuticals are approved to treat SCD, these are non-curative and provide modest benefit to a fraction of patients with SCD. Allogeneic HSCT may offer a cure, but only for the small minority of patients with an available matched donor. Overall, treatment of patients with SCD remains an unmet medical need.

SCD is caused by the presence of HbS due to a point mutation substituting valine for glutamic acid in the sixth codon of the beta-globin gene. When deoxygenated, HbS polymerizes, creating rigid fibrils that lead to occlusion of blood vessels and hemolysis, as illustrated in Figure 1.

Figure 1. Schema Depicting Pathophysiology of Sickle Cell Disease

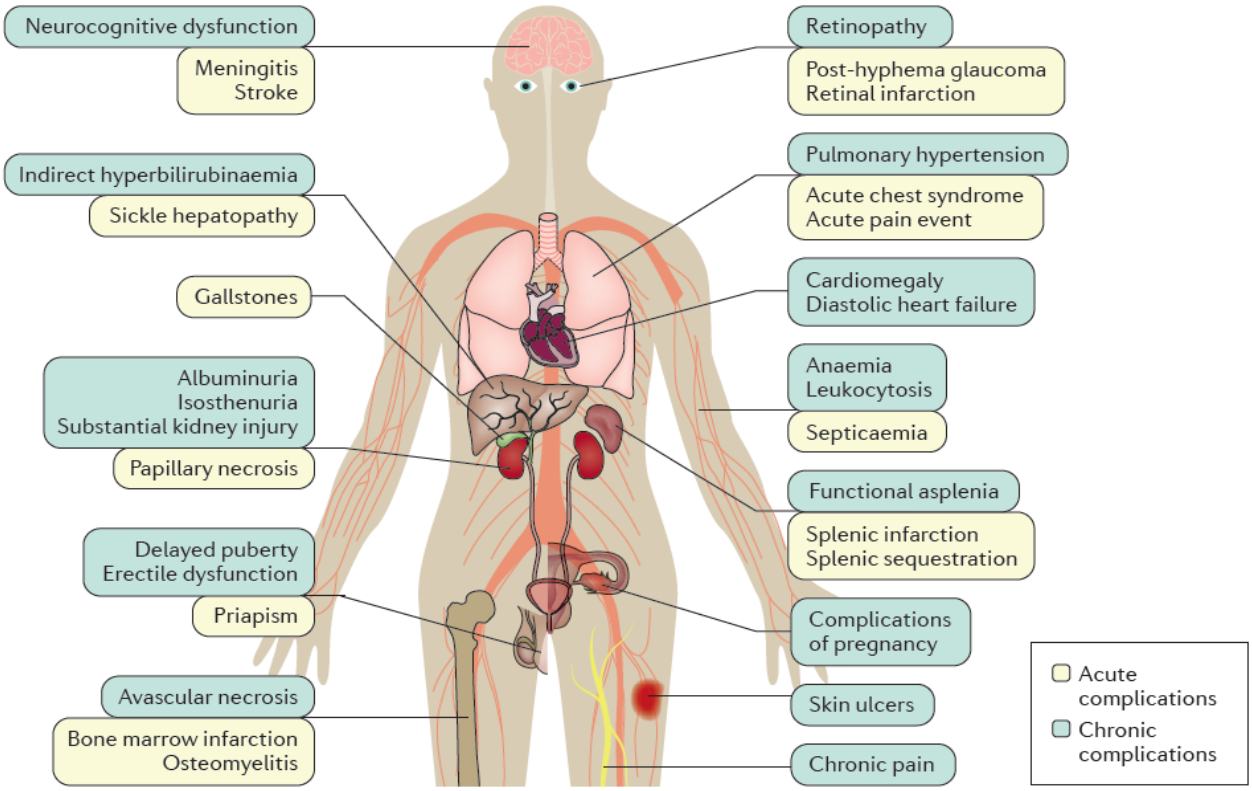


Source: Steinberg Martin H. Fetal-like Hemoglobin in Sickle Cell Anemia. N Engl J Med 2022; 386:689-691
DOI:10.1056/NEJMe2119760

The disease is characterized by debilitating manifestations such as pain, anemia, strokes, retinopathy, pulmonary hypertension, and chronic ischemic damage to organs such as kidney, liver, and bone. Early splenic infarction leaves young children particularly susceptible to overwhelming sepsis. Please see Figure 2.

[13] As noted in footnote 1, hemoglobin-C disease is excluded from consideration in this document.
[14] Pecker, LH, BA Schaefer, and L Luchtman-Jones, 2017, Knowledge insufficient: the management of haemoglobin SC disease, Br J Haematol, 176(4):515-526.
[15] 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.

Figure 2. Depiction of Acute and Chronic Complications of Sickle Cell Disease



Source: Kato, GJ, FB Piel, CD Reid, MH Gaston, K Ohene-Frempong, L Krishnamurti, WR Smith, JA Panepinto, DJ Weatherall, FF Costa, and EP Vichinsky, 2018, Sickle cell disease, Nat Rev Dis Primers, 4:18010.

The SCD population suffers from lifelong morbidity and early mortality. Management of SCD includes penicillin prophylaxis, transcranial Doppler monitoring, pain control, and exchange or simple RBC transfusions. Pharmacologic agents such as hydroxyurea (approved in 1998) and L-glutamine, voxelotor, and crizanlizumab (approved over the last 6 years) have modestly improved the outcomes of many patients with SCD. As late as the 1970s, mortality of U.S. children diagnosed with SCD was poor, with approximately half dying before adulthood.[16] While adults with SCD continue to experience substantially shorter survival compared to unaffected peers, the survival of children with SCD has dramatically improved due to clinical advancements developed over the past 50 years, and nearly all children in developed countries are now expected to survive into adulthood.[17] The only available curative therapy is allogeneic HSCT; however, this procedure carries significant risks and fewer than 20% of patients with SCD have an appropriate human leukocyte antigen-matched donor.[18] Consequently, SCD treatment remains an unmet medical need.

[16] Scott, RB, 1970, Health care priority and sickle cell anemia, Jama, 214(4):731-734.
[17] 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.
[18] Mentzer, WC, S Heller, PR Pearle, E Hackney, and E Vichinsky, 1994, Availability of related donors for bone marrow transplantation in sickle cell anemia, Am J Pediatr Hematol Oncol, 16(1):27-29.

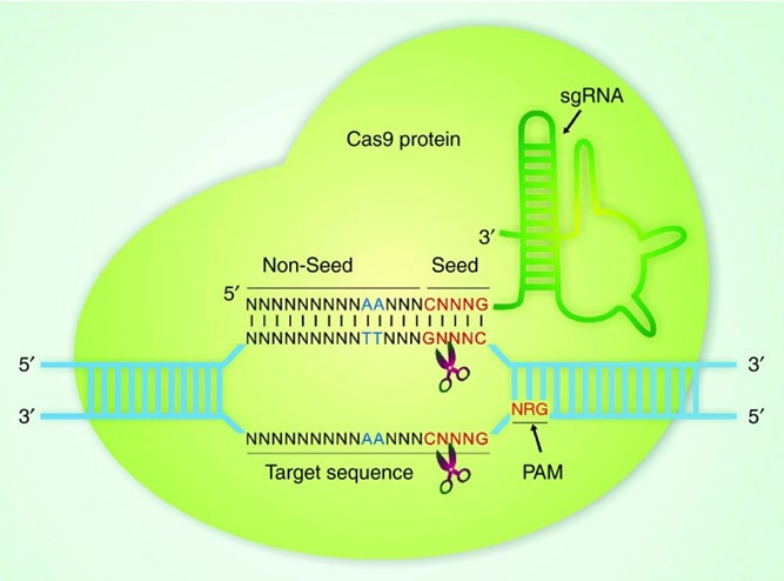
2.2 Pertinent Drug Development Information

2.2.1 Genome Editing

FDA defines human genome editing as a process by which DNA sequences are added, deleted, altered, or replaced at specified location(s) in the genome of human somatic cells, ex vivo or in vivo, or by using nuclease-dependent or nuclease-independent genome editing technologies.[19] Nuclease-dependent technologies, such as CRISPR/Cas, introduce site-specific breaks in the DNA, which is intended to result in modification of the DNA sequence at the target site. CRISPR Cas systems are naturally occurring microbial defense mechanisms that have been engineered to cleave genomic DNA. The CRISPR Cas system utilized by the Applicant in the generation of exa-cel is the *Streptococcus pyogenes* Cas9 system. Cas9 is directed to precisely cleave a specific target double-stranded DNA sequence, using a gRNA sequence. Naturally, the gRNA consists of a CRISPR RNA that is responsible for target complementarity, and a trans-activating RNA that is required to bind the CRISPR RNA to the Cas9 protein. These gRNA sequences were combined into a single gRNA (sgRNA) for the production of exa-cel. The Cas9 protein binds to the sgRNA to form a ribonucleoprotein complex. Once in the nucleus of a cell, the Cas9 nuclease is guided by the sgRNA through the genomic DNA seeking a protospacer adjacent motif (PAM). If such a PAM is adjacent to a DNA sequence complementary to the sgRNA, the sgRNA specifically binds to the DNA sequence of interest enabling the Cas9 enzyme to introduce a double-strand DNA break (DSB) three to four nucleotides upstream of the PAM sequence. Following cleavage, endogenous DNA repair mechanisms repair the cut, typically through non-homologous end-joining, which often introduces insertions or deletions of bases (referred to as “indels”). In the case of exa-cel, the generation of an indel results in the reduction of transcription factor binding and ultimately decreased levels of the target protein production.

[19] Draft guidance for industry *Human Gene Therapy Products Incorporating Human Genome Editing* (March 2022).

Figure 3. A Cartoon Depiction of the Target Genomic DNA and the Components of the CRISPR/Cas9 sgRNA Genome Editing System



Source: Zhang, XH, LY Tee, XG Wang, QS Huang, and SH Yang, 2015, Off-target Effects in CRISPR/Cas9-mediated Genome Engineering, Mol Ther Nucleic Acids, 4(11):e264.
Abbreviations: CRISPR/Cas9, clustered regularly interspaced short palindromic repeats associated 9 nucleases; NRG, neuregulin; PAM, protospacer adjacent motif; sgRNA, single guide RNA.

2.2.2 Off-Target Editing Assessment

One of the main concerns related to genome editing technology is risk of cleavage of genomic DNA at unintended sites due to imperfect pairing between the gRNA and the target DNA sequence. A subset of these imperfectly paired sites can be cleaved by the Cas9 endonuclease resulting in unintended edits across the genome.[20,21,22,23] These sites can tolerate up to 6-mismatches between the gRNA and the genomic DNA. Since unintended edits can disrupt gene expression if present in the coding or regulatory DNA sequences, it is critical that the specificity of the gRNA be thoroughly screened to ensure off-target genome editing is minimized. Unintended genome editing can be screened using quantitative bioinformatics methods that uses genome-wide NGS data.

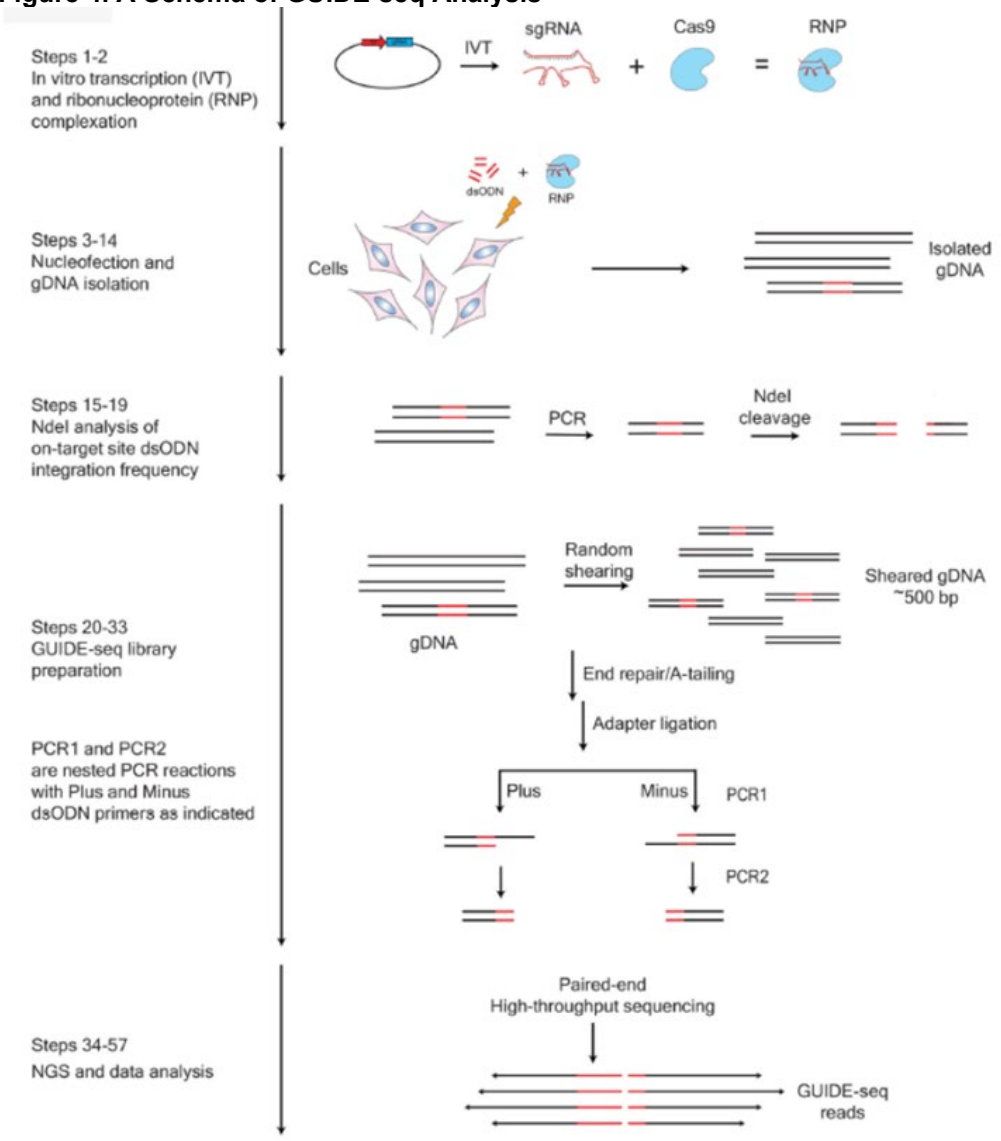
Several methods for genome-wide off-target editing detection have been developed that can be broadly classified into three categories: in silico, cellular, and biochemical. The in silico off-target detection methods rely on computational algorithms developed to scan the human

[20] Hsu, PD, DA Scott, JA Weinstein, FA Ran, S Konermann, V Agarwala, Y Li, EJ Fine, X Wu, O Shalem, TJ Cradick, LA Marraffini, G Bao, and F Zhang, 2013, DNA targeting specificity of RNA-guided Cas9 nucleases, Nat Biotechnol, 31(9):827-832.
[21] Fu, Y, JA Foden, C Khayter, ML Maeder, D Reyon, JK Joung, and JD Sander, 2013, High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells, Nat Biotechnol, 31(9):822-826.
[22] Pattanayak, V, S Lin, JP Guilinger, E Ma, JA Doudna, and DR Liu, 2013, High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity, Nat Biotechnol, 31(9):839-843.
[23] Cong, L, FA Ran, D Cox, S Lin, R Barretto, N Habib, PD Hsu, X Wu, W Jiang, LA Marraffini, and F Zhang, 2013, Multiplex genome engineering using CRISPR/Cas systems, Science, 339(6121):819-823.

reference genome and identify sites that are homologous to the user-provided gRNA.[24] The off-target loci nominated using these methods rely on the user-provided mismatch criteria between the gRNA and target site. This may not reflect the true in vivo off-target editing events arising due to lower sequence similarity between gRNA and the native genome. Since the in silico algorithms rely on sequence homology, increasing the mismatch criteria may result in a long list of off-target sites with a large number of false positives (FPs). Performing confirmatory testing using targeted sequencing can become prohibitively expensive. Furthermore, in silico methods fail to capture the cellular complexity arising from variable genome accessibility associated with the cell type specific chromatin landscape. Therefore, in silico off-target detection should be complemented using either a cellular or biochemical off-target detection method. The Applicant chose to use a cellular off-target analysis method which relies on sequencing genomic material from Cas9-gRNA edited cells. These methods can be very sensitive in identifying high confidence off-target sites that are cell-type specific. GUIDE-seq, which stands for Genome-wide Unbiased Identification of DSBs Enabled by Sequencing, is one of the most widely used cellular off-target detection methods. GUIDE-seq relies on the integration of double-stranded oligodeoxynucleotide (dsODN) tags at the site of DSBs in genome edited cells. The GUIDE-seq experiment entails editing cells with Cas9 and gRNA in the presence of dsODN (workflow depicted, Figure 4).

[24] Guo, C, X Ma, F Gao, and Y Guo, 2023, Off-target effects in CRISPR/Cas9 gene editing, Front Bioeng Biotechnol, 11:1143157.

Figure 4. A Schema of GUIDE-seq Analysis



Source: Malinin, NL, G Lee, CR Lazzarotto, Y Li, Z Zheng, NT Nguyen, M Liebers, VV Topkar, AJ Iafrate, LP Le, MJ Aryee, JK Joung, and SQ Tsai, 2021, Defining genome-wide CRISPR-Cas genome-editing nuclease activity with GUIDE-seq, Nat Protoc, 16(12):5592-5615.
Note: Cells of interest is incubated with RNP complex and dsODN. The genomic DNA of the edited cells is extracted, and library of the sheared DNA is prepared for sequencing and analyzed by the GUIDE-seq bioinformatics pipeline
Abbreviations: Cas9, CRISPR-associated 9 nucleases; dsODN, double-stranded oligodeoxynucleotides; DSB, double-strand DNA break; gDNA, genomic DNA; GUIDE-seq, Genome-wide Unbiased Identification of DSBs Enabled by Sequencing; IVT, in vitro transcription; NGS, next-generation sequencing; PCR, polymerase chain reaction; RNP, ribonucleoprotein; sgRNA, single guide RNA.

The genomic DNA from the edited cells is then isolated for NGS analysis to identify and quantify on- and off-target edit sites. Since DSBs can occur spontaneously in cells, CRISPR/Cas9-independent events may appear as FPs in GUIDE-seq assays. To exclude FPs, the GUIDE-seq experiment routinely includes analysis of genomic DNA from control cells that are incubated with dsODN only. The NGS data from these control samples are used to exclude spontaneous DSBs arising independently of CRISPR/Cas9 editing. While cellular off-target analysis methods

are effective in off-target identification, these methods can also be difficult to apply for certain cell types due to toxicity associated with dsODN.

In summary, off-target analysis tools can be broadly categorized into three methods:

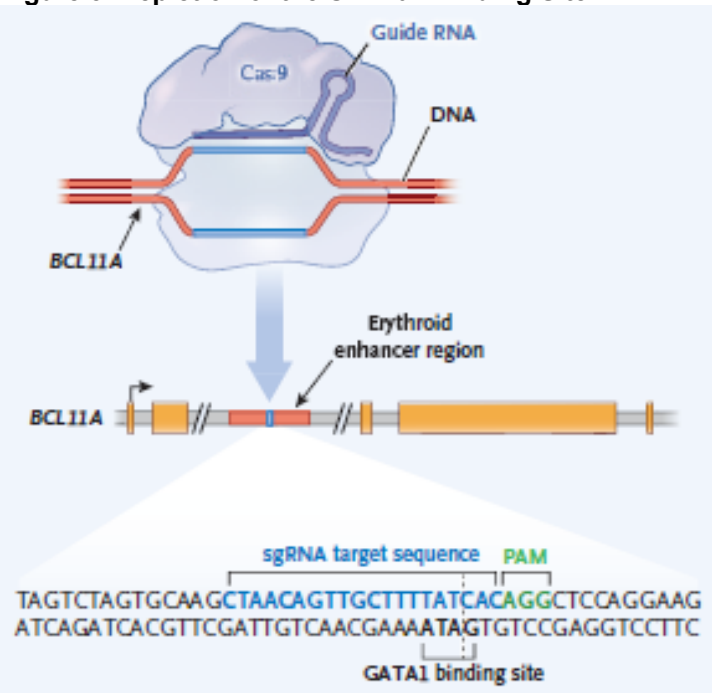
- In silico methods use computational algorithms to identify off-target sites based on sequence homology. These methods are straightforward to implement but can miss true off-target sites occurring in cells due to base pairing between gRNA and genomic DNA, with more mismatches than the allowed threshold.
- The biochemical off-target analysis methods use genomic DNA of cells that are edited with CRISPR/Cas9 to identify potential off-target edits. These methods are useful in those cases where the cells intended for genome editing are difficult to culture and/or are present in small fractions in the body. However, these methods do not account for cell type specificity, nor do they capture the cellular complexity arising from inherent DNA repair processes and the chromatin landscape of the cells.
- The cellular GUIDE-seq analysis uses integration of dsODN tags as a readout for on- and off-target edits present in cells. This technique can be challenging to implement due to the toxicity associated with culturing cells in the presence of dsODN. Since each off-target analysis method has its unique set of strengths and limitations, a comprehensive analysis would entail testing of a suitable sample size and using a combination of two or more orthogonal methods to adequately assess product safety.[25]

2.2.3 Product Description

Exa-cel is comprised of autologous hematopoietic stem cells (HSCs) genome edited *Streptococcus pyogenes* Cas9 and *SPY101* sgRNA targeting a binding site of the transcription factor *GATA1* in the erythroid lineage-specific enhancer region of the *BCL11A* gene, suspended in a cryopreservation medium. The genome editing is intended to disrupt *GATA1* binding at this site, thus lowering *BCL11A* expression specifically in erythroid cells (Figure 5). This reduction in *BCL11A* leads to increased gamma globin and thereby increased HbF. HbF is known to be therapeutic in individuals with SCD who also experience hereditary persistence of HbF.[26]

[25] Draft guidance for industry *Human Gene Therapy Products Incorporating Human Genome Editing* (March 2022).
[26] Steinberg, MH, 2020, Fetal hemoglobin in sickle cell anemia, Blood, 136(21):2392-2400.

Figure 5. Depiction of the *SPY101* Binding Site



Source: Frangoul, H, D Altshuler, MD Cappellini, YS Chen, J Domm, BK Eustace, J Foell, J de la Fuente, S Grupp, R Handgretinger, TW Ho, A Kattamis, A Kernytsky, J Lekstrom-Himes, AM Li, F Locatelli, MY Mapara, M de Montalembert, D Rondelli, A Sharma, S Sheth, S Soni, MH Steinberg, D Wall, A Yen, and S Corbacioglu, 2021, CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia, N Engl J Med, 384(3):252-260.
Abbreviations: *BCL11A*, B-cell lymphoma/leukemia 11A; Cas9, CRISPR- associated 9 nucleases; PAM, protospacer adjacent motif; sgRNA, single guide RNA.

2.2.4 Exa-cel sgRNA

SPY101 is a 100-base pair sgRNA with the sequence depicted in Figure 6. Methylated 2' ribosyl hydroxyl groups and thiolated phosphate linkages have been incorporated at both terminal ends to inhibit degradation by nucleases.

Figure 6. *SPY101* Sequence

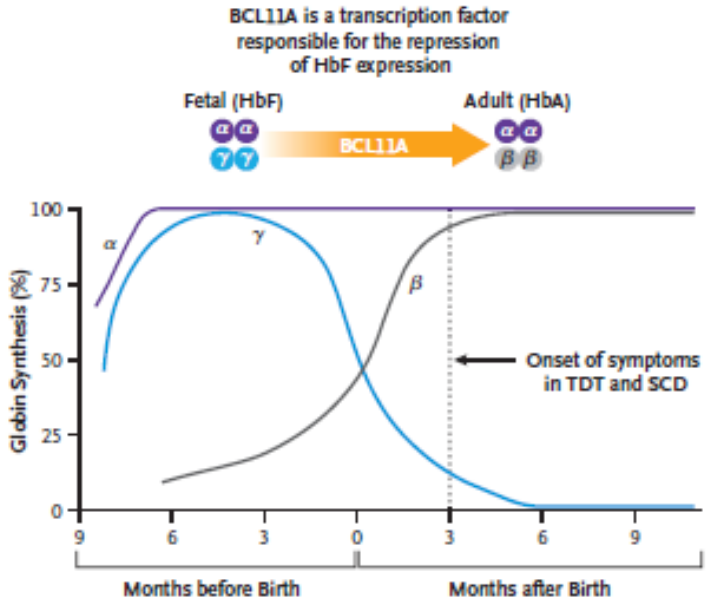
OMe-rC OMe-rU* OMe-rA* ACA GUU GCU UUU AUC ACG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC OMe-rU* OMe-rU* OMe-rU* U*

Source: Frangoul, H, D Altshuler, MD Cappellini, YS Chen, J Domm, BK Eustace, J Foell, J de la Fuente, S Grupp, R Handgretinger, TW Ho, A Kattamis, A Kernytsky, J Lekstrom-Himes, AM Li, F Locatelli, MY Mapara, M de Montalembert, D Rondelli, A Sharma, S Sheth, S Soni, MH Steinberg, D Wall, A Yen, and S Corbacioglu, 2021, CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia, N Engl J Med, 384(3):252-260.
* Phosphorothioate positions

2.2.5 Physiologic Role of *BCL11A* and Mechanism of Action of Exa-cel

HbF consists of two alpha and two gamma globin chains. Adult hemoglobin consists of two alpha and two beta globin chains. During late fetal development, gamma globin expression is repressed leading to the transition from HbF to adult hemoglobin after birth. *BCL11A* is a zinc finger–containing transcription factor that represses gamma globin expression in erythroid cells. A graphical depiction of this transition is presented in Figure 7.

Figure 7. Diagram of the Transition From HbF to HbA



Source: Frangoul, H, D Altshuler, MD Cappellini, YS Chen, J Domm, BK Eustace, J Foell, J de la Fuente, S Grupp, R Handgretinger, TW Ho, A Kattamis, A Kernytsky, J Lekstrom-Himes, AM Li, F Locatelli, MY Mapara, M de Montalembert, D Rondelli, A Sharma, S Sheth, S Soni, MH Steinberg, D Wall, A Yen, and S Corbacioglu, 2021, CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia, N Engl J Med, 384(3):252-260
Abbreviations: BCL11A, B-cell lymphoma/leukemia 11A; HbA, adult hemoglobin; HbF, fetal hemoglobin; SCD, sickle cell disease, TDT, transfusion-dependent β-thalassemia.

SPY101 targets Cas9-mediated cleavage to the *GATA1* binding site in the erythroid lineage-specific enhancer region of the *BCL11A* gene. Introduction of indels into this region of the genome of HSCs disrupts *GATA1* binding following erythroid differentiation, thus lowering *BCL11A* expression specifically in erythroid cells. This reduction in *BCL11A* expression alleviates the *BCL11A*-mediated block of gamma globin expression. Increased gamma globin expression results in increased HbF. The upregulation of HbF is predicted to lessen the symptoms of SCD following engraftment of the subject's own genome edited HSCs.

2.2.6 Product Manufacturing

To produce exa-cel, HSCs are collected from patients by apheresis after mobilization with plerixafor. CD34+ cells are then isolated using a closed, automated, micro-bead system (CliniMACS Prodigy System, Miltenyi Biotec). Purified CD34+ cells are then electroporated with *SPY101* containing Cas9 ribonucleoproteins using the MaxCyte Gen2 GT electroporator. After electroporation, the cells are briefly incubated in the defined culture medium prior to being washed and cryopreserved.

3. Summary of Efficacy

Subjects treated with exa-cel in Study 121 expressed increased HbF within approximately 3 months post infusion. Of 30 efficacy analysis eligible subjects treated with exa-cel, 29 reached the primary efficacy endpoint of freedom from sVOCs for 12 months from infusion of exa-cel

(VF12), and 28/29 VF12 achievers remained free of sVOCs for a mean duration of 22.3 (standard deviation [SD] 7.2) months. One subject died approximately 9 months after exa-cel infusion; if this subject is included as not having achieved VF12, then 29/31 (93.5%) of subjects met the primary endpoint.

3.1 Sources of Data for Efficacy

Data in support of exa-cel efficacy comes from the ongoing Study 121 and the long-term follow-up (LTFU) Study 131. Study 121 is a multinational, single arm, Phase 1/2/3 study to evaluate the safety and efficacy of a single dose of exa-cel in subjects 12 to 35 years old with severe SCD with recurrent VOCs. The target population is 45 subjects.

Study 121

Objectives

The primary objective was to evaluate the safety and efficacy of a single dose of exa-cel in subjects with severe SCD. Secondary objectives were to assess the effects of infusion of exa-cel on disease-specific events and clinical status, and to quantify genome editing efficiency.

Key Inclusion Criteria

Subjects 12 to 35 years old with genotypes β^S/β^S, β^S/β^0, or β^S/β^+ and severe SCD defined by the occurrence of at least two of the following events each year during the 2-year period before screening:

1. Acute pain event that required a visit to a medical facility and administration of pain medications (opioids or intravenous non-steroidal anti-inflammatory drugs) or RBC transfusions.
2. Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever.
3. Priapism lasting >2 hours and requiring a visit to a medical facility.
4. Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in Hb concentration of ≥2 g/dL.

Key Exclusion Criteria

1. Availability of human leukocyte antigen matched marrow donor, history of prior HSCT, active infections, cytopenias, organ dysfunction (liver/lung/ heart).
2. History of abnormal transcranial Doppler results for subjects 12 to 18 years of age, history of Moyamoya disease, or any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk to the subject; or having baseline HbF concentration >15%.

3. >10 unplanned hospitalizations or emergency department visits due to SCD in the year before screening that, in the opinion of investigator, are consistent with significant chronic pain rather than acute pain crises.

Efficacy Endpoints

The primary efficacy endpoint was the proportion of subjects who had achieved VF12, defined as absence of sVOCs for a period of 12 months at any point on Study 121 following exa-cel infusion. The evaluation of VF12 started 60 days after last RBC transfusion for post-transplant support or SCD management.

Key Secondary Efficacy Endpoint

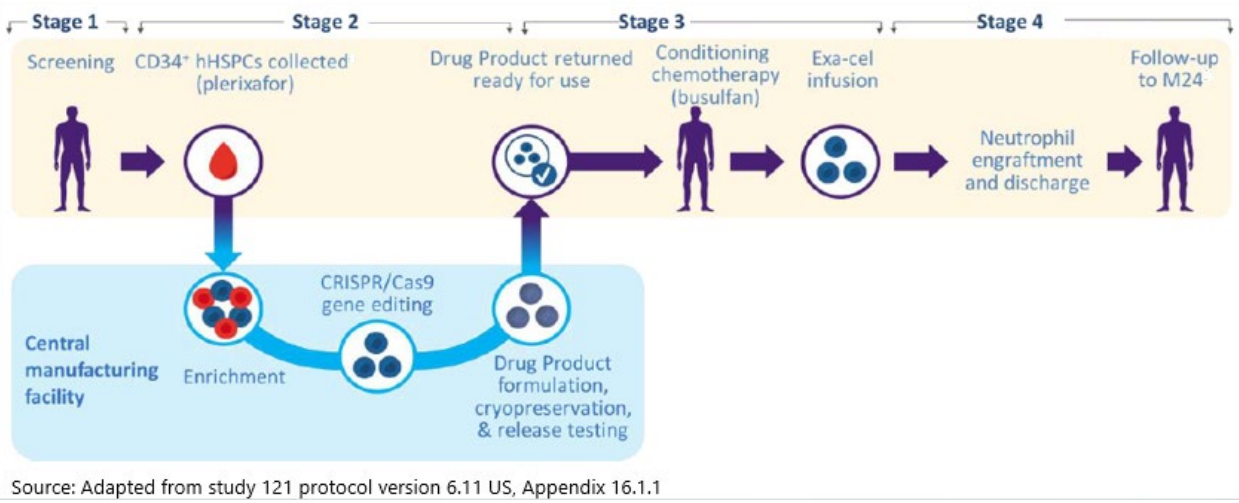
The proportion of subjects who achieved freedom from inpatient hospitalization for sVOCs sustained for at least 12 months (HF12) after exa-cel infusion. The evaluation of HF12 started 60 days after last RBC transfusion for post-transplant support for SCD management.

Secondary Efficacy Endpoints

1. The duration of freedom from sVOCs in subjects who had achieved VF12.
2. The relative reduction from baseline in annualized rate of sVOCs up to 24 months starting after Month 12 post exa-cel infusion for subjects who had not achieved VF12.
3. The proportion of subjects with sustained HbF ≥20% at the time of analysis for at least 3 months, 6 months, or 12 months was evaluated starting 60 days after last RBC transfusion for post-transplant support or SCD management.

The study conduct is depicted in Figure 8.

Figure 8. Schema of Study 121 Conduct



Source: Adapted from Study 121 protocol version 6.11 US, Appendix 16.1.1
Abbreviations: CD34+, cluster of differentiation 34+; CRISPR/Cas9, clustered, regularly interspaced, short palindromic repeats-associated 9 nucleases; hHSPC, hematopoietic stem and progenitor cell; M, month.

LTFU Study 131

Study 131 is an ongoing, global, multisite, rollover study designed to evaluate the long-term safety and efficacy of exa-cel in subjects who received exa-cel in Studies 121 (SCD) and 111 (TDT). All subjects who received exa-cel and who complete or discontinue Study 121 are eligible and are asked/encouraged to participate in this study. Study 131 participants undergo evaluation of most of the assessments and endpoints described in Study 121 at a reduced frequency until 15 years from exa-cel infusion.

Analysis Populations

- Enrolled Set: N=63. A subpopulation which includes enrolled subjects who signed informed consent and met eligibility criteria.
- Safety Analysis Set: At time of IA2, N=58. A subpopulation which contains those Enrolled Set subjects who started mobilization.
- Full Analysis Set (FAS): At time of IA2, N=42. A subset which includes those Enrolled Set subjects who were infused with exa-cel.
- Primary Efficacy Set (PES): At time of IA2, N=20. A subset of FAS subjects who had at least 16 months follow-up after exa-cel infusion, thus efficacy analysis eligible. The evaluation of VF12 started 60 days after the last RBC transfusion.

A subsequent submission with a data lock date of June 14, 2023 provided additional data, increasing the FAS population to 44 and PES to 30 subjects.

Demographics

The median (range) age of the 44 subjects in the FAS was 20 (12 to 34) years, with 12 adolescents <18 years old and 32 adults. Forty (90.9%) subjects had *βs/βs* genotype, and 45.5% were female.

Baseline Characteristics

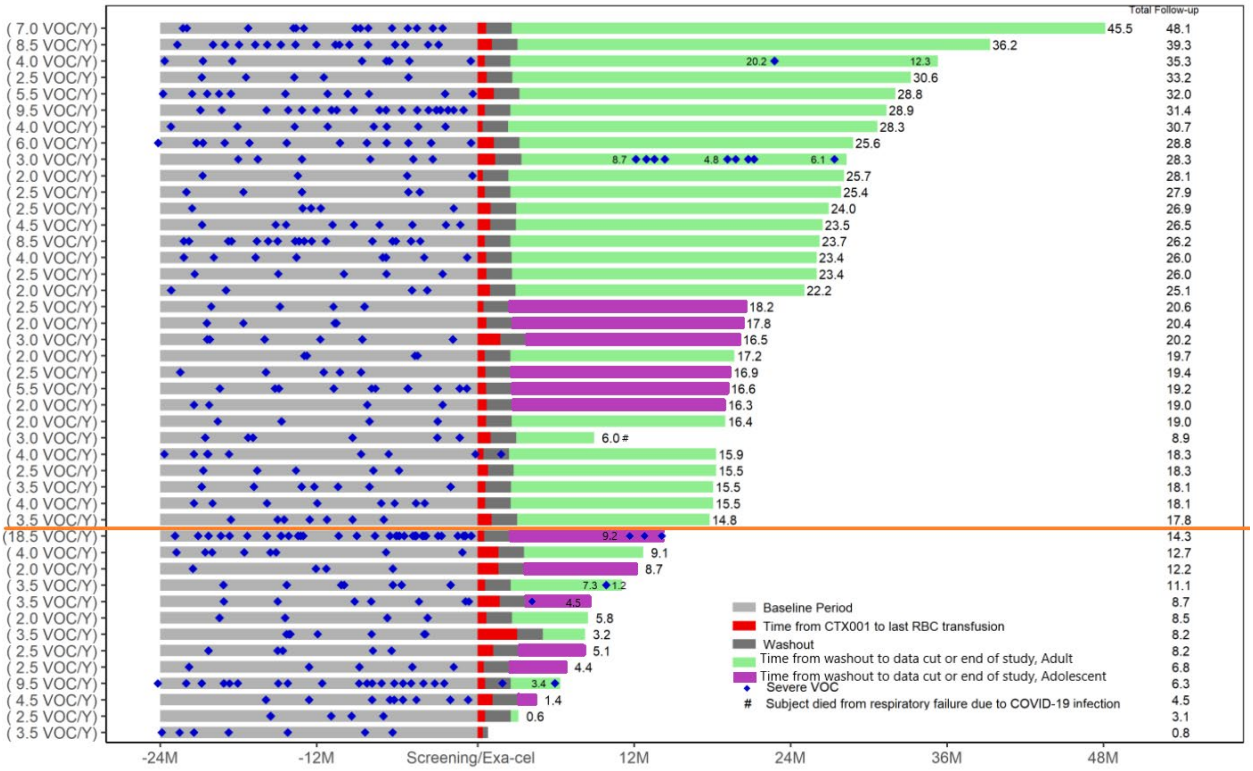
Subjects had a mean (range) historical annualized rate of sVOCs of 4.1 (2.0 to 18.5), with a mean (range) historical annualized rate of inpatient hospitalizations for sVOCs of 2.7 (0.5 to 9.5) and mean (range) annualized duration of inpatient hospitalizations for sVOCs of 19.7 (2.0 to 136.5) days. Subjects had a mean (range) of 11.3 (0 to 86.1) historical annualized units of RBCs transfused related to SCD. Their median HbF concentration was 5% (range 0 to 14.7%), with a median total Hb of 9.4 g/dL (5.7 to 12.6).

Efficacy Results

Of the 30 subjects evaluable for efficacy analysis, 29 (96.7%) achieved the primary efficacy endpoint of VF12. According to the pre-specified statistical analysis plan, study success was based on a third planned interim analysis conducted at a significance level of 0.0054,

corresponding to a one-sided 99.46% confidence interval of (78%, 100%). Of the 29 subjects achieving VF12, 28 had 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 HF12, which is a key secondary efficacy endpoint of Study 121. The primary efficacy endpoint data are depicted in Figure 9. All 6 adolescents (100%) included in the PES achieved VF12, and had between 19.0 and 20.6 months of follow up. One additional adolescent failed to achieve VF12 by protocol definition, but is not included in PES due to a follow up < 16 months. This subject has 14.3 months of follow up and experienced three sVOCs between Month 11.1 to 14.1.

Figure 9. Historical and After Exa-cel sVOCs and sVOC Free Duration in All Dosed Subjects, FAS



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.

The clinical efficacy endpoints were supported by pharmacodynamic endpoints demonstrating that in subjects with SCD, the mean allelic editing in CD34+ cells of the bone marrow was ≥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 through Month 42.

Subjects produced increased fetal and total hemoglobin, with total mean (SD) Hb levels of 12.0 (1.3) g/dL at Month 3, which increased and were maintained with a mean ≥11.1 g/dL from Month 6 to the date of data lock.

The mean (SD) proportion of total Hb composed of HbF (%) was 36.8% (7.8%) at Month 3 and was maintained at generally ≥40% from Month 6 over the duration of follow-up.

Figure 9 The above data support evidence of efficacy of exa-cel. It is important to note that the data come from a single primary study which was uncontrolled and small. Such single-arm studies are subject to various biases that can limit confidence in the magnitude of the treatment effect. However, given the strongly positive results, FDA does not believe that the study design limitations call the efficacy of exa-cel into question.

4. Summary of Issues for the Advisory Committee

4.1 Safety Issue

4.1.1 CRISPR/Cas9 Off-Target Analysis and Safety Assessment of Exa-cel

For an adequate safety assessment, off-target analysis should account for human genetic variation or heterogeneity in the intended population. This would require using a database that has adequate representation of target population genome sequence variations. The Applicant used variant information in the 1,000 genomes project database that has sequencing data from 2,504 individuals. By analyzing variants with allele frequency of >1% in at least 1 continental group in this database, they were able to identify 50 additional off-target candidate sites. The reference database used by the Applicant contains WGS data from 661 individuals of the target population. In this group, sequencing data from 61 individuals from the southwestern United States were included. It is not clear if this limited amount of WGS data would sufficiently capture variants present in the target population. Insufficient sequencing data may impede the identification of relevant variants contributing to off-target editing.

For the cellular off-target analysis, the Applicant used three samples from healthy donors and three samples from subjects with SCD of African American ethnicity. Given the impact of the SCD on HSPC function, which can potentially change the chromatin landscape and can impact off-target editing, the merits of using healthy donor samples for such analysis is not clear. Additionally, it is not clear if the small number of samples used in the cellular GUIDE-seq off-target analysis is sufficient to adequately assess off-target editing in exa-cel.

4.1.1.1 In Silico Analysis Off-Target Analysis Data for Exa-cel

The Applicant used three publicly available in silico algorithms to nominate potential off-target sites for the sgRNA *SPY101* (Figure 6) based on its homology to the reference sequence. The

three algorithms used were (b) (4) Each of these algorithms uses a homology-based off-target search that is unique to the tool. (b) (4) uses the (b) (4) sequence alignment and ranks the off-target sites by (b) (4). The (b) (4) method considers mismatches closer to the PAM sequence (shown as the seed sequence in Figure 3). Mismatches in this region have been shown to decrease the likelihood of an off-target edit. In summary, (b) (4) can be inclusive of up to 5 mismatches but would not identify off-targets with more than (b) (4) mismatches in the seed region. (b) (4) uses the (b) (4) and searches for off-targets with either (b) (4) mismatches and (b) (4) indel or with just (b) (4) mismatches. (b) (4) uses the (b) (4) algorithm and provides users with additional off-target identification options and scores such as cutting frequency determination.

For off-target nomination, the Applicant first used 5 mismatch criteria when implementing (b) (4) and used default mismatch criteria when implementing (b) (4). They also used suboptimal PAMs such as NGA, NAA, NCG, NGC, NTG, and NGT with 4 mismatches to nominate off-targets. From this analysis, a total of 5,007 loci were identified as potential homology-based off-target sites. Confirmatory testing was performed using a hybrid capture library that targeted these 5,007 sites. The hybrid capture sequencing used CD34+ HSPCs from four healthy donors with either CRISPR/Cas9/*SPY101* and control editing HSPCs. The Applicant did not provide data on the demographic information for the source of the samples used in the confirmatory testing by hybrid capture sequencing.

Statistical significance for confirmatory testing was set at ≥1% indel frequency difference between edited and control samples. A summary of the hybrid-capture assessment of off-target sites nominated from the in silico method is presented in Table 1 (see below). No editing was observed at any of the 5,007 loci nominated by in silico analysis when assessed by hybrid capture sequencing.

(b) (4)

Table 1. Hybrid Capture Characteristics and Results for Sites Identified Through Sequence Homology

Number of Mismatches: PAM Types	Number of Off-Target Sites Identified	Number of Sites With Sufficient Quality	Total Sites Confirmed by Hybrid Capture
5: NGG, NAG 4: NGA, NAA, NCG, NGC, NTG, NGT	5007	4340	0

Source: Table 6: Hybrid Capture Characteristics and Results for Sites Identified through Sequence Homology (ctxsr-015.pdf, BLA 125787 Amendment 001 2017)
Abbreviation: PAM, protospacer adjacent motif.

Subsequently, the Applicant performed another in silico analysis in which they excluded loci with >3 mismatches. This filtering resulted in the nomination of 171 off-target loci that were subjected to confirmatory testing with hybrid capture sequencing using the genomic DNA samples from *SPY101* edited CD34+ HSPCs from 4 healthy donors. The details of the healthy donor-derived HSPCs used in hybrid capture sequencing experiment is presented in Table 2. Three of the hybrid capture sequencing samples were from the individuals of Hispanic ethnicity and one from African American ethnicity. Statistical significance for confirmatory testing was set at ≥0.2% indel frequency difference between edited and control samples.

Table 2. Metadata for Samples Used for Hybrid Capture Experiments

Donor ID	Disease Status	Age (Years)	Sex	Race/Ethnicity
Donor 1	Healthy	29	Female	Hispanic
Donor 2	Healthy	27	Male	Hispanic/Latino
Donor 3	Healthy	31	Male	Hispanic
Donor 4	Healthy	35	Male	African American

Source: Table 1: Metadata for Hybrid Capture Experiments (responses-to-fda-bioinformatics-ir5.pdf, Amendment 0042, 2023)

In this analysis, there were no statistically significant off-target editing events observed at any of the off-targets nominated using in silico analysis. The summary of the hybrid-capture assessment of off-target candidate sites nominated from the in silico analysis is presented in Table 3 (see below).

Table 3. Hybrid Capture Characteristics and Results for Sites Identified Through Sequence Homology

Number of Mismatches: PAM Types	Number of Off-Target Sites Identified	Total Sites Confirmed by Hybrid Capture
3: NGG, NAG, NGA, NAA, NCG, NGC, NTG, NGT	171	0

Source: Table 7: Hybrid Capture Characteristics and Results for Sites Identified through Sequence Homology (res-ind-042.pdf, BLA 125787 Amendment 001 2018)
Abbreviation: PAM, protospacer adjacent motif.

To address the impact of human genetic variation on off-target activity, the Applicant used the variant information from the 1,000 genomes project. This database used by the Applicant has WGS data from 2,504 individuals who are divided into five continental groups: Africa, Americas, East Asia, Europe, and South Asia. The total number of samples present in each of these continental groups is presented in Table 4.

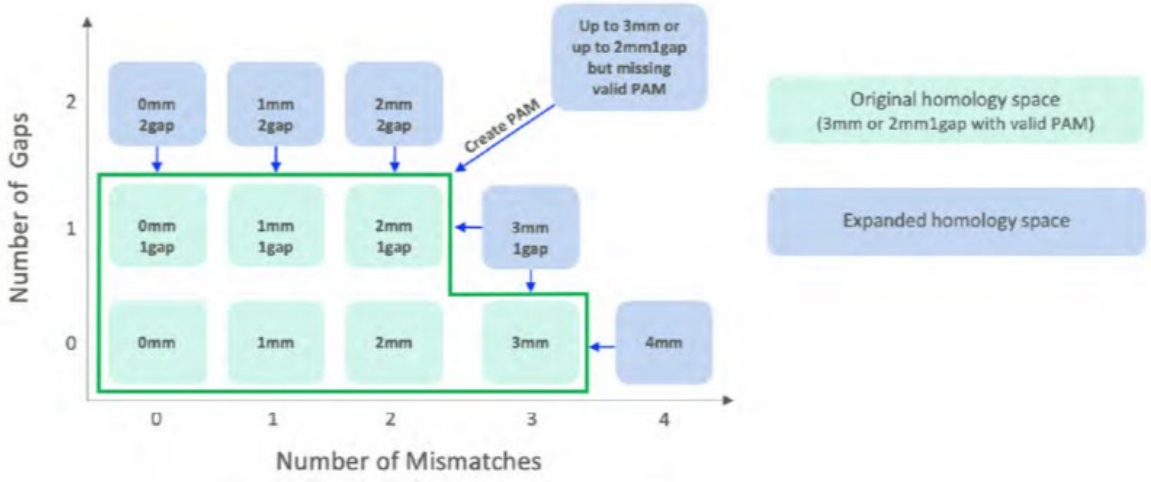
Table 4. Population Ancestry Breakdown of 2504 Individuals in the 1000 Genomes Project

Continental Group	Total Samples
Africa	661
Americas	347
East Asia	504
Europe	503
South Asia	489

Source: Table 4: Population Ancestry Breakdown of 2504 Individuals in Phase 3 of the 1000 Genomes Project (response-to-bioinformatics-ir-6.pdf, BLA 125787 Amendment 047 2023)

Specifically, they stated that they used version 151 of database of single nucleotide polymorphisms variant information that includes ~83 million single nucleotide polymorphisms, insertions, and deletions. The Applicant used two different allele frequency cutoffs while performing variant aware off-target nomination. First, they tested ~7 million variants that have a global allele frequency of >10% in the 1,000 genomes project database. They then implemented a variant-aware off-target search that used sites with 4 mismatches that would turn into a 3-mismatch site upon inclusion of the variant nucleotide. Alternately, they included sites that, upon inclusion of the variant nucleotide, would result in creation of a PAM sequence with either 3 mismatches or up to 2 mismatches and 1 gap. A schema of the variant-aware homology search is provided in Figure 10 (see below).

Figure 10. Schematic of Variant-Aware Homology Search



Source: r264.pdf, BLA125787 Amendment 001
Abbreviation: PAM, protospacer adjacent motif.

From this analysis, they identified nine additional off-target loci with non-canonical PAM sequences. Eight of these off-targets were intergenic and one was intronic. The off-targets and the associated variant allele frequency and annotation are reported in Table 5.

Table 5. Candidate Off-Target Regions Identified Through Computational Variant-Aware Homology Search at 10% Minor Allele Frequency Threshold

Off-Target ID	Genomic Location	Variant Allele Frequency
homva_ot1	Intergenic	42.2%
homva_ot2	Intron	46.2%
homva_ot3	Intergenic	37.7%
homva_ot4	Intergenic	97.1%
homva_ot5	Intergenic	73.7%
homva_ot6	Intergenic	21.5%
homva_ot7	Intergenic	41.0%
homva_ot8	Intergenic	43.4%
homva_ot9	Intergenic	12.4%

Source: Table 10: Candidate Off-Target Regions Identified Through Computational Variant-Aware Homology Search at 10% Minor Allele Frequency Threshold (r264.pdf, BLA 125787 Amendment 001 2022)

In the next analysis, they included ~21 million variants from the 1,000 genomes project database with >1% allele frequency cutoff in at least one of the five continental groups. From this analysis, they identified 41 additional candidate off-target loci. Table 6 lists a subset of 20 off-target loci that were either intronic or exonic, their respective annotations, and the continental group that has an allele frequency >1%. Seven of these off-target loci arose from variants present at frequency >3% in the African continental group.

Table 6. Candidate Off-Target Regions Identified Through Computational Variant-Aware Homology Search at 1% Minor Allele Frequency Threshold

Site Coordinates	Annotation	Gene Name	Homology	Global Variant Frequency	Population-Specific Variant Frequency	Population With Variant Frequency >1%
chr1:100152737-100152758	Intron	LRRC39	2mm	0.44%	1.49%	EUR
chr1:100152738-100152758	Intron	LRRC39	2mm	0.44%	1.49%	EUR
chr10:17367608-17367629	Intron	ST8SIA6	2mm,1gap	0.80%	3.03%	AFR
chr11:108331858-108331879	ExonIntron	ATM	2mm,1gap	0.46%	1.74%	AFR
chr11:112196597-112196618	Intron	BCO2	2mm,1gap	0.24%	1.19%	EAS
chr12:4651564-4651586	Intron	NDUFA9	2mm,1gap	0.30%	1.13%	AFR
chr12:4651563-4651586	Intron	NDUFA9	2mm,1gap	0.30%	1.13%	AFR
chr12:61748091-61748111	Intron	FAM19A2	2mm,1gap	36.30%|0.12%	47.28%|0.45%	Global,|None
chr12:64064594-64064615	Intron	SRGAP1	2mm,1gap	1.44%|1.44%	5.3%|5.3%	Global,AFR|Global,AFR
chr12:104862519-104862540	Intron	SLC41A2	2mm,1gap	1.38%	4.99%	Global,AFR
chr13:46667776-46667797	Intron	LRCH1	2mm,1gap	0.72%	3.27%	SAS
chr5:131306754-131306775	Intron	CDC42SE2	2mm,1gap	0.20%	1.02%	SAS
chr5:147417885-147417906	Intron	DPYSL3	2mm,1gap	2.34%	8.62%	Global,AFR
chr6:86944935-86944957	Intron	HTR1E	2mm,1gap	25.06%|6.81%	37.22%|13.84%	Global|Global
chr8:53720818-53720839	Intron	ATP6V1H	2mm,1gap	0.44%	1.66%	AFR
chr9:16618034-16618056	Intron	BNC2	2mm,1gap	0.84%|0.10%	3.03%|0.38%	AFR|None
chrX:85346594-85346615	Intron	POF1B	2mm,1gap	0.53%	2.09%	EUR

Site Coordinates	Annotation	Gene Name	Homology	Global Variant Frequency	Population-Specific Variant Frequency	Population With Variant Frequency >1%
chr5:148425480-148425502	Intron	FBXO38	3mm	0.92%	3.48%	AFR
chr7:33483352-33483374	Intron	BBS9	3mm	0.50%	1.82%	AFR
chr7:70395236-70395258	Intron	AUTS2	3mm	0.20%	1.01%	AMR

Source: Table 13-1 41: Candidate Off-target Sites Identified Through Computational Variant-aware Homology Search at 1% Minor Allele Frequency Threshold (response-to-bioinformatics-ir-6.pdf, BLA 125787 Amendment 047 2023)
Note: “ExonIntron” refers to an off-target cut site located 5-bp away from the intron-exon boundary.
For off-target loci where two genetic variants were included for homology, the frequency and population information are separated by “|.”
Abbreviations: AFR, Africa; AMR, Americas; EAS, East Asia; EUR, Europe; SAS, South Asia.

However, a closer look at the samples included in the African continental subgroup showed that the individuals that were sampled in these groups were predominantly from the western or eastern regions of the African continent. A small amount of the sequencing data was from individuals who were either from the southwest United States (N=61) or the Caribbean in Barbados (N=96). A breakdown of the number of samples from these regions is provided in Table 7.

Table 7. Population Ancestry Breakdown of 661 African Continental Group Individuals in the 1000 Genomes Project

Population-Region	Population Code	Number of Individuals
Esan in Nigeria	AFR	99
Gambian in Western Division, Mandinka	AFR	113
Luhya in Webuye, Kenya	AFR	99
Mende in Sierra Leone	AFR	85
Yoruba in Ibadan, Nigeria	AFR	108
African Caribbean in Barbados	AFR/AMR	96
People with African ancestry in Southwest United States	AFR/AMR	61

Source: Table 4: Population Ancestry Breakdown of 2504 Individuals in Phase 3 of the 1000 Genomes Project (response-to-bioinformatics-ir-6.pdf, BLA 125787 Amendment 047 2023)
Abbreviations: AFR, Africa; AMR, Americas.

African American individuals make up 13.6% of the U.S. population,[31] which is about ~45 million people.[32] SCD is the most common genetic disorder affecting 1 in 500 African Americans and an estimated 100,000 people make up the patient population.[33] Given the large number of patients in United States who are the intended target population for this drug, it is not clear if the sequencing information from the limited number of individuals captured in the 1,000 genomes project reference database (see Table 8) would sufficiently capture variants that may contribute to an off-target locus.

Finally, the Applicant reported 50 new off-target loci by including variant information from the 1,000 genomes project database. Of these, 20 loci annotated to 18 genes whose intronic/exonic locations were identified as potential off-target loci. One of these off-target loci was 5-bp from the intron-exon junction of *ATM* gene that is known to cause a rare neurodegenerative disease in subjects homozygous for null mutations and is associated with increased cancer risk in subjects with heterozygous disease-causing mutations. Since an edit near the exon-intron junction can potentially disrupt *ATM* function, the Applicant provided a risk assessment for this locus. They stated that the concerning off-target locus uses a non-canonical PAM with a gap

[31] Bureau, USC, Quick Facts, accessed September 14, 2023, https://www.census.gov/quickfacts/fact/table/US/RHI225222.
[32] Christine Tamir, 2021, The Growing Diversity of Black America, Pew Research Center, accessed September 14, 2023, https://www.pewresearch.org/social-trends/2021/03/25/the-growing-diversity-of-black-america/#:~:text=46.8%20million%20people%20in%20the%20U.S.%20identify%20as%20Black,-How%20we%20did.
[33] Sedrak, A and NP Kondamudi, 2023, Sickle Cell Disease, StatPearls, Treasure Island (FL): StatPearls Publishing, Copyright © 2023, StatPearls Publishing LLC.

that has been shown to have very low likelihood of off-target editing. Additionally, they stated that this variant has an allele frequency of 1.74% in the target population and would likely be present in individuals as one copy and rarely as two copies. The remaining 17 genes encode for proteins involved in GTPase signaling, mitochondria, DNA repair, etc. Since CRISPR/Cas9 editing has been shown to result in large deletions,[34] such editing events in the intronic loci can potentially disrupt these genes' function. However, the impact of the loss of any of the 17 genes function on HSPCs has not been evaluated. The Applicant stated that they performed confirmatory testing of these newly nominated 50 off-targets in 1 SCD and 2 TDT donor cells and demonstrated no significant off-target editing at these loci. Since the off-target loci were identified in the presence of a variant, confirmatory testing should be performed in samples that are known to carry variants of interest. The applicant reported the presence of 13 variants in at least one sample that was used for confirmatory testing. Of this, three variants were present in just one sample. Since the remaining 37 variants were not present in the samples, an absence of editing in the confirmatory testing may not necessarily rule out off-target editing at these sites in individuals that harbor these alternate alleles.

4.1.1.2 Cellular Off-Target Analysis Data for Exa-cel

The Applicant used GUIDE-seq to identify candidate off-targets of *SPY101* in healthy donor, TDT donor, and SCD donor-derived CD34+ HSPCs. In the healthy CD34+ HSPCs study, the Applicant used cells from 1 healthy donor and tested the effects of various dsODN concentrations spanning over (b) (4) µM and (b) (4) incubation times (b) (4) hours. At dsODN concentrations (b) (4) µM, they observed low cell viability (<36%) and a high number of off-target loci that is indicative of DNA degradation in the dying cells. Hence, the Applicant reported off-target loci identified with an optimal dsODN concentration, where they observed >87% cell viability (see Table 8 below).

Table 8. Sites Identified in *SPY101* GUIDE-seq

dsODN Concentration	Treatment Time	Cell Viability (%)	Total Off-Target Sites
(b) micro molar	(b) (4)	88	(b) (4)
(b) micro molar	(b) (4)	94	(b) (4)

Source: Table 7: Sites Identified in SPY101 GUIDE-seq (ctxsr-016.pdf, Amendment 001 2017)
Abbreviations: dsODN, double-stranded oligodeoxynucleotides; DSB, double-strand DNA break; GUIDE-seq, Genome-wide Unbiased Identification of DSBs Enabled by Sequencing.

In subsequent experiments, the Applicant used optimized dsODN concentrations where they observed >70% cell viability. For the two additional healthy donor-derived CD34+ HSPCs, the Applicant performed GUIDE-seq with a lower dsODN concentration compared to the previous experiment and reported adequate number of on-target reads. They also reported sixteen and five off-target loci in each healthy donor sample tested (see Table 9 below).

[34] Park, SH, M Cao, Y Pan, TH Davis, L Saxena, H Deshmukh, Y Fu, T Treangen, VA Sheehan, and G Bao, 2022, Comprehensive analysis and accurate quantification of unintended large gene modifications induced by CRISPR-Cas9 gene editing, Sci Adv, 8(42):eabo7676.

Table 9. Sufficient dsODN Incorporation by On-Target Read Count

Donor ID	Disease Status	Cell Viability (%)	dsODN Concentration	On-Target Read Count	Total Off-Target Sites
Donor 5	Healthy	71	(b) (4) micro molar	12,095	16
Donor 6	Healthy	75	(b) (4) micro molar	11,336	5

Source: Table 8: Sufficient dsODN Incorporation by On-Target Read Count; Table 9: Sites Identified in SPY101-RNP Treated GUIDE-seq Samples Across Two Donors (res-ind-041.pdf, BLA 125787 Amendment 001).
Abbreviation: dsODN, double-stranded oligodeoxynucleotides.

No common off-target loci were identified between these three healthy donor cell samples (results presented in Table 9 and Table 10). The healthy donor cells were treated with two different concentrations of dsODN, which could potentially interfere with identification of common off-target loci. The Applicant, however, reasoned that the use of different dsODN concentrations was acceptable since they were able to identify adequate numbers of on-target reads in all the experiments irrespective of the dsODN concentrations used. Combining all the off-target loci from the healthy donor HSPC studies, a total of 52 off-target loci were identified and were tested using hybrid capture sequencing.

The four healthy donor samples used in hybrid capture sequencing experiment were different from the donors used in the GUIDE-seq experiment and their details are presented in Table 2. Three of these samples were from the donors of Hispanic race/ethnicity and one sample was from a donor of African American race/ethnicity. Statistical significance for confirmatory testing was set at ≥0.2% indel frequency difference between edited/treated and control samples.

From this analysis, none of the potential 52 off-target loci tested showed statistically significant off-target editing (see Table 10 below).

Table 10. Hybrid Capture Characteristics and Results for Regions Identified Through GUIDE-seq

Samples Used for GUIDE-seq & dsODN Concentrations	Number of Off-Target Sites Identified from All Experiments	Total Sites Confirmed by Hybrid Capture
Donor 5 ((b) (4) micro molar) Donor 6 ((b) (4) micro molar) Donor 7 ((b) (micro molar)	52	0

Source: Table 8: Hybrid Capture Characteristics and Results for Regions Identified through GUIDE-seq (res-ind-042.pdf, BLA 125787 Amendment 001)
Abbreviations: dsODN, double-stranded oligodeoxynucleotides; DSB, double-strand DNA break; GUIDE-seq, Genome-wide Unbiased Identification of DSBs Enabled by Sequencing.

Next, the Applicant performed GUIDE-seq analysis on six CD34+ HSPC samples. Specifically, they edited CD34+ HSPCs from three subjects with SCD and three subjects with TDT in the presence of an optimal concentration of dsODN where 70 to 83% cell viability was observed.[35]

In this experiment, the Applicant reported an adequate number of on-target reads and a high on-target editing rate for each sample (see Table 11**,** columns 3 and 5, respectively). A total of

[35] Vertex Pharmaceuticals Inc, Table 14: Live Cell Number and Viability of Each Condition, r263.pdf, BLA125787 Amendment 001.

64 off-targets were identified across 6 samples using GUIDE-seq analysis (see Table 11, column 4).

Table 11. Editing Rates and On-target Read Counts for GUIDE-Seq Experiments

Samples	dsODN Concentration	On-Target Read Count	Number of Off-Target Sites	On-Target Editing Rate (%)
SCD1	(b) (4) micro molar	16,508	12	83.8
SCD2	(b) (4) micro molar	28,879	13	93.5
SCD3	(b) (4) micro molar	20,857	17	93.6
TDT1	(b) (4) micro molar	23,468	5	92
TDT2	(b) (4) micro molar	23,938	11	92.6
TDT3	(b) (4) micro molar	18,807	6	87.7

Source: Table 6: Editing Rates and On-target Read Counts for GUIDE-Seq Experiments (nonclin-info-amend.pdf, Amendment 12, 2023); Table 4: GUIDE-seq in Cells from Six Patient Samples (nonclin-info-amend.pdf, Amendment 14, 2023)
Abbreviations: dsODN, double-stranded oligodeoxynucleotides; DSB, double-strand DNA break; GUIDE-seq, Genome-wide Unbiased Identification of DSBs Enabled by Sequencing; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia.

They performed hybrid capture sequencing to confirm off-target loci identified in this study as well as from the preceding computational analysis and GUIDE-seq analysis performed on healthy donor cells. A summary of all the non-overlapping loci identified in the prior experiments is presented in Table 12.

Table 12. Summary of Regions Included in Hybrid Capture Analysis

Cellular Off-Target Studies	Total Sites Identified
GUIDE-seq in healthy donor derived HSPCs	52
In silico off-target nomination	171
Off-targets nominated using variant-aware homology search	9
GUIDE-seq in subject HSPCs	64

Source: Table 4: Summary of Regions Included in Hybrid Capture Analysis for Each Patient (r264.pdf, BLA125787 Amendment 001); Table 4: GUIDE-seq in Cells from Six Patient Samples (nonclin-info-amend.pdf, Amendment 14, 2023)
Abbreviations: DSB, double-strand DNA break; GUIDE-seq, Genome-wide Unbiased Identification of DSBs Enabled by Sequencing; HSPC, hematopoietic stem and progenitor cell.

For hybrid capture sequencing, they reported a median on-target reads range of 20,000 to 35,000, and on-target editing rates ranged from 60 to 72% in the edited/treated samples (shown in Table 13, columns 2 and 3, respectively). They also reported five loci with >0.2% indel frequency in treated experimental samples compared to controls (Table 13, column 4) that annotated to the centromeric region of chr3.

Table 13. Median On-Target Coverage for Each Sample and Sites Identified in Hybrid Capture Sequencing

Samples	Median On-Target Read Counts	Median On-Target Editing Frequency (%)	Number of Sites From Hybrid Capture
SCD1	20278.5	71.8	2
SCD2	22075.5	66.2	0
SCD3	22004.5	71.9	1
TDT1	30457.0	67.2	0
TDT2	34790.0	60.8	1
TDT3	26328.5	71.7	1

Source: Table 6: Median On- and Off-Target Coverage for Each Patient; Table 7: On-Target Editing Rates for Each Patient; Table 8: Summary of Significant Results for Each Patient (r264.pdf, BLA125787 Amendment 001)
Abbreviations: SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia.

We noted that the on-target editing frequency in the hybrid capture experiment is lower than the on-target editing frequency observed in GUIDE-seq for the same six samples. The lower on-target editing frequency can interfere with optimal editing at off-target loci as they occur at a much lower rate. The Applicant stated that their hybrid capture sequencing was performed at high depth to enable detection of low frequency off-target edits.

In a separate analysis, the Applicant used control GUIDE-seq sequencing data from eight samples: six were from SCD and TDT donors (same as described above), and two were from healthy donors. In this analysis, they reported editing at 13 loci in control samples and reasoned these DSBs were naturally occurring hotspots that are independent of CRISPR/Cas9 editing activity.

Finally, the Applicant attributed the off-targets identified in these samples as likely FPs. To support this conclusion, they reanalyzed the GUIDE-seq data using matched control sequencing data and applied the FP filtering step from GUIDE-seq. From this reanalysis, they reported that all the off-targets loci identified by GUIDE-seq across 6 samples were removed after FP filtering (Table 14).

Table 14. Results From GUIDE-seq With and Without "False Positive Filtering"

Samples	dsODN Concentration	Number of Off-Target Sites	Number of Filtered Off-Target Sites
SCD1	(b) (4) micro molar	12	0
SCD2	(b) (4) micro molar	13	0
SCD3	(b) (4) micro molar	17	0
TDT1	(b) (4) micro molar	5	0
TDT2	(b) (4) micro molar	11	0
TDT3	(b) (4) micro molar	6	0

Source: Table 1: Results from GUIDE-seq Nomination in Patient Off-target Study (R263) With and Without "False Positive Filtering" (scd-mcm-followup.pdf, BLA125787 Amendment 040)
Abbreviations: dsODN, double-stranded oligodeoxynucleotides; DSB, double-strand DNA break; GUIDE-seq, Genome-wide Unbiased Identification of DSBs Enabled by Sequencing; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia.

Together, from the GUIDE-seq experiments, manual analysis of the off-target loci, and by applying FP filtering, the Applicant concluded that no evidence for *SPY101* off-target editing was identified.

4.1.2 Safety Summary

The Applicant used two orthogonal methods for off-target assessment of sgRNA *SPY101*: in silico sequence-homology-based off-target nomination and the cellular GUIDE-seq analysis. In the first analysis, the Applicant used three different in silico analysis tools and a mismatch criterion of either 3 or 5 and identified 171 or 5,007 potential off-target loci, respectively. These tools used the hg38 human reference genome for scanning off-target sites. To account for human sequence variability in the target population, the Applicant used variant information from the 1,000 genomes project database and selected a subset of variants with allele frequency >1% in at least 1 continental group in the 1,000 genomes project database. This analysis yielded 50 additional off-target loci. The 1,000 genomes project database used by the

Applicant contained variant data from WGS of 661 individuals of the target population. This group contained sequencing data from 61 individuals who were from the southwest United States. The remaining samples were from individuals who were from either the eastern or western regions of the African continent. Given the limited amount of sequencing data from the intended population, it is not clear how comprehensively the variants in the target population were assessed.

In the second analysis, the Applicant performed two GUIDE-seq experiments, one using three healthy donor-derived CD34+ HSPCs and another using three SCD donor-derived CD34+ HSPCs and three TDT donor-derived CD34+ HSPCs. They performed confirmatory testing of the GUIDE-seq loci using hybrid capture in four independent healthy donor-derived CD34+ HSPCs and in the same three SCD donor-derived CD34+ HSPCs. Several studies have shown that SCD is an inflammation-inducing condition and can activate stress response processes.[36,37] Oxidative stress has been shown to contribute to chromatin modification in SCD[38]. SCD also impacts HSPC function and can alter HSPC lineage.[39,40] These changes have the potential to impact the chromatin landscape of SCD donor derived CD34+ HSPCs. Since chromatin accessibility can influence off-target activity,[41,42,43] it is not clear if GUIDE-seq analysis of healthy donor derived CD34+ HSPCs can adequately capture potential off-target editing occurring in patient cells. However, availability of SCD donor cells can be limited and should also be considered. The Applicant used a total of four samples that were from donors of African American ethnicity. Three of these samples were from SCD donors that were used in the GUIDE-seq experiment and hybrid capture sequencing experiment, and one sample was from healthy donor that was used in the hybrid capture sequencing experiment. Given the limited number of SCD samples that

[36] Hoppe, CC, 2014, Inflammatory mediators of endothelial injury in sickle cell disease, Hematol Oncol Clin North Am, 28(2):265-286.
[37] Hebbel, RP and GM Vercellotti, 2021, Multiple inducers of endothelial NOS (eNOS) dysfunction in sickle cell disease, Am J Hematol, 96(11):1505-1517.
[38] Xi, C, J Pang, W Zhi, CS Chang, U Siddaramappa, H Shi, A Horuzsko, BS Pace, and X Zhu, 2023, Nrf2 sensitizes ferroptosis through l-2-hydroxyglutarate-mediated chromatin modifications in sickle cell disease, Blood, 142(4):382-396.
[39] Leonard, A, A Bonifacino, VM Dominical, M Luo, JJ Haro-Mora, S Demirci, N Uchida, FJ Pierciey, Jr., and JF Tisdale, 2019, Bone marrow characterization in sickle cell disease: inflammation and stress erythropoiesis lead to suboptimal CD34 recovery, Br J Haematol, 186(2):286-299.
[40] Javazon, EH, M Radhi, B Gangadharan, J Perry, and DR Archer, 2012, Hematopoietic stem cell function in a murine model of sickle cell disease, Anemia, 2012:387385.
[41] Kim, D and JS Kim, 2018, DIG-seq: a genome-wide CRISPR off-target profiling method using chromatin DNA, Genome Res, 28(12):1894-1900.
[42] Guo, C, X Ma, F Gao, and Y Guo, 2023, Off-target effects in CRISPR/Cas9 gene editing, Front Bioeng Biotechnol, 11:1143157.
[43] Hinz, JM, MF Laughery, and JJ Wyrick, 2016, Nucleosomes Selectively Inhibit Cas9 Off-target Activity at a Site Located at the Nucleosome Edge, J Biol Chem, 291(48):24851-24856.

were used in the cellular off-target analysis, it is not clear if the GUIDE-seq analysis adequately assessed the potential off-target editing by exa-cel.[44]

4.2 Risk Mitigation

Should this product be approved, the Applicant has proposed both routine pharmacovigilance and a postmarketing safety study (VX22-290-101) for postmarketing safety monitoring for exa-cel. Routine pharmacovigilance will include AE reporting in accordance with 21 CFR 600.80. The proposed postmarketing safety study (VX22-290-101) is a prospective observational cohort registry study, which will compare 250 SCD patients who received exa-cel to those who received allogenic HSCT. Patients will be followed for 15 years, evaluating safety outcomes including malignancy and hematologic disorders, end organ damage/dysfunction, disease severity, survival, and transplant-related complications.

The Applicant's pharmacovigilance plan and proposed postmarketing safety study are under review at this time, and FDA will provide recommendations to the Applicant as needed.

5. References

References can be found in the footnotes.

[44] Shah, NC, S Bhoopatiraju, A Abraham, E Anderson, M Andreansky, M Bhatia, S Chaudhury, GDE Cuvelier, K Godder, M Grimley, G Hale, N Kamani, D Jacobsohn, A Ngwube, AL Gilman, J Skiles, LC Yu, and S Shenoy, 2022, Granulocyte Colony-Stimulating Factor Is Safe and Well Tolerated following Allogeneic Transplantation in Patients with Sickle Cell Disease, Transplant Cell Ther, 28(3):174.e171-174.e175.