

I concur with this memo. C. Saeui 12/20/23.

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PRODUCT: CASGEVY (exagamglogene autotemcel; exa-cel; CTX001)

APPLICANT: Vertex Pharmaceuticals Incorporated

PROPOSED INDICATION: Treatment of Transfusion Dependent Beta-Thalassemia (TDT)

PHARM/TOX REVIEWER: Cheauyun (Theresa) Chen
 PHARM/TOX BRANCH CHIEF: Christopher Saeui (acting for Abigail Shearin)
 PRODUCT (CMC) REVIEWERS: Anna Kwilas, Jessica Chery, Elena Gubina, Eric Levenson, Brain Stultz, Zhaohui Ye
 CLINICAL REVIEWERS: Kavita Natrajan (efficacy), Umer (Muhammad) Choudhry (safety)
 BIOINFORMATICS REVIEWER: Komudi Singh
 PROJECT MANAGER: Danielle Bauman
 OFFICE DIRECTOR of Iwen Wu
 PHARM/TOX:

EXECUTIVE SUMMARY:

CASGEVY is a cellular suspension consisting of autologous CD34+ human hematopoietic stem progenitor cells (hHSPCs) that are genetically modified *ex vivo* by CRISPR-Cas9-mediated genome editing technology. The genome editing is carried out by electroporation (EP) of CD34+ HSPCs with a Cas9-SPY101-ribonucleoprotein (SPY101-RNP) genome editing complex containing recombinant Cas9 protein and a BCL11A/SPY101 single guide RNA (SPY101 gRNA) that targets the *B-Cell Lymphoma/Leukemia 11A (BCL11A)* gene in the GATA1 transcription factor-binding site. The SPY101-RNP mediated genome editing creates a double

strand break (DSB) at the target site in the *BCL11A* gene, followed by repair of the DSB by the non-homologous end joining (NHEJ) pathway, and formation of insertions-deletions (indels). The disruption of the *BCL11A* gene, a repressor of γ -globin gene transcription, leads to reactivation of γ -globin expression in erythroid precursors and fetal hemoglobin (HbF) formation in erythroid cells which is intended to improve erythroid function in patients with TDT.

The *in vitro* pharmacology studies conducted with CD34+ cells from healthy donors edited using the SPY101-RNP used in the manufacture of CASGEVY showed editing at the target genomic locus of the BCL11A/GATA1 binding site, with genome editing frequencies ranging from 60% to 92%. Subsequent upregulation of γ -globin transcripts and HbF levels were observed compared to unedited cells and on-target editing frequencies were stable during erythroid differentiation. There were no significant editing-related changes to cell viability, cell growth, erythroid differentiation, enucleation, and distribution profile across various lineage progenitor subpopulations compared to unedited CD34+ hHSPCs. Edited CD34+ hHSPCs from healthy donors and patients with TDT showed similar on-target editing frequencies and upregulation of γ -globin transcript and HbF protein.

The *in vivo* pharmacology of SPY101-RNP edited CD34+ hHSPCs from healthy donors was evaluated in irradiated NOD/SCID/IL2Rgnull (NSG) mice. Engraftment of transplanted cells and the on-target editing frequencies were evaluated. Single IV administration of edited CD34+ hHSPCs at 1×10^6 cells/mouse resulted in similar levels of chimerism of CD34+ hHSPCs in whole blood, bone marrow, and spleen, as well as multilineage differentiation to B-, T-, and myeloid cells in whole blood, bone marrow, and spleen in studies of 16- and 20-weeks duration compared to unedited cells. At 16 weeks post-administration, engrafted cells in NSG mice demonstrated >90% on-target editing frequencies in whole blood, bone marrow, and spleen. Erythroid progenitor cells differentiated from bone marrow-engrafted cells isolated at 16 weeks post-administration had an average of 90% on-target editing frequency.

An *in vivo* pharmacokinetic study of SPY101-RNP edited CD34+ hHSPCs from healthy donors was conducted in irradiated NSG mice. Human DNA was detected in most of the examined tissues in mice that received a single IV administration of 1×10^6 cells/mouse, with the highest levels detected in the bone marrow, followed by spleen, blood, lung, liver, and kidney. Low levels of human DNA were detected at the injection site, heart, mammary gland, jejunum, pancreas, brain, and skeletal muscle. Human DNA levels were minimal to below the limit of quantification in the prostate, uterus, ovary, and testis at 8 weeks post-administration. Editing frequencies of $87.4 \pm 1.5\%$ were observed in the spleen and bone marrow at 8- and 20-weeks post-administration.

An *in vivo* toxicology and tumorigenicity study of SPY101-RNP edited CD34+ hHSPCs from healthy donors was conducted in irradiated NSG mice. Mice received single IV administration of 1×10^6 cells/mouse and were followed for 20 weeks. There were no significant adverse findings or tumor formation.

The potential for SPY101-RNP mediated off-target editing and chromosomal aberrations was evaluated for CD34+ hHSPCs from healthy donors, patients with sickle cell disease (SCD), and patients with TDT. No evidence of off-target editing was observed by hybrid capture sequencing

analyses on the candidate off-target sites nominated by in-silico and GUIDE-seq analyses. No chromosomal aberrations for SPY101-RNP edited CD34+ hHSPCs from healthy donors were observed based on karyotyping, long-range PCR sequencing, and hybrid capture sequencing analyses.

Developmental and reproductive toxicity studies and carcinogenicity studies were not conducted with CASGEVY. These studies are not warranted based on the characteristics and safety profile of the product.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies in the pharmacology-toxicology studies for CASGEVY. There are no outstanding requests for additional nonclinical data for evaluation of CASGEVY. The nonclinical data provided in the BLA submission support the approval of this biologics license application.

Formulation and Chemistry:

CASGEVY (exagamaglogene autotemcel [exa-cel], formerly known as CTX001), consists of autologous CD34+ hHSPCs from patients diagnosed with SCD or TDT that are isolated from mobilized peripheral blood (mPB), using G-CSF alone, plerixafor alone, or G-CSF and plerixafor in combination, and modified *ex vivo* by CRISPR-Cas9-mediated genome editing. The genome editing is carried out using i) Cas9 protein - a wild type (WT) *Streptococcus pyogenes* Cas9 nuclease (spCas9) protein with (b) (4) and ii) SPY101 gRNA that targets the GATA1 transcription factor-binding site of the enhancer region of the *BCL11A* gene, which are (b) (4) to form an SPY101-RNP complex, followed by delivery into CD34+ hHSPCs by EP. CASGEVY is a cellular suspension formulated in (b) (4) cryopreservation medium containing 5% dimethyl sulfoxide (DMSO) and Dextran-40.

Abbreviations

AIDE	Amplicon Indel Detection
BCL11A	B-cell lymphoma/leukemia 11A
(b) (4)	(b) (4)
BM	Bone marrow
Cas9	CRISPR-associated protein 9
CD	Cluster of differentiation
(b) (4)	(b) (4)
CMP	Common myeloid progenitors
CRISPR	Clustered regularly interspaced short palindromic repeats
DMSO	Dimethyl sulfoxide
DSB	Double-strand break
dsOND	Double-stranded oligodeoxynucleotide
EGFP	Enhanced green fluorescent protein

EGFP-RNP	EGFP gRNA Cas9 ribonucleoprotein
EP	Electroporation
(b) (4)-RNP	(b) (4) gRNA Cas9 ribonucleoprotein
FACS	Fluorescence-activated cell sorting
GATA1	GATA binding protein 1 (globin transcription factor 1)
GLP	Good Laboratory Practice
GMP	Granulocyte-macrophage progenitors
gRNA	Guide RNA
GUIDE-seq	Genome-wide Unbiased Identification of DSBs by sequencing
Hb	Hemoglobin
HbA	Adult hemoglobin
HbF	Fetal hemoglobin
hHSPC	Human hematopoietic stem and progenitor cell
HPFH	Hereditary persistence of fetal hemoglobin
(b) (4)	
HSPC	Hematopoietic stem and progenitor cell
(b) (4)	
Indel	Insertion deletion
(b) (4)	
LT-HSC	Long-term repopulating hematopoietic stem cells
MEP	Megakaryocyte-erythrocyte progenitors
MLP	Multipotent lymphoid progenitors
MPP	Multipotent progenitors
mPB	mobilized peripheral blood
mRNA	Messenger RNA
NGS	Next generation sequencing
NHNJ	Non-homologous end joining
(b) (4)	
OECD	Organization for Economic Cooperation and Development
PBS	Phosphate Buffered Saline
PBMC	Peripheral blood mononuclear cell
POC	Proof-of-concept
(b) (4)	
RBCs	Red blood cells
RNP	Ribonucleoprotein
rRNA	Ribosomal RNA
SCC	Single cell colony
SCD	Sickle cell disease
SCID	Severe combined immunodeficiency
SD	Standard deviation
(b) (4)-RNP	(b) (4) gRNA Cas9 ribonucleoprotein
sgRNA	Single-guide RNA (also named gRNA)
SPY101-RNP	SPY101 gRNA Cas9 ribonucleoprotein
(b) (4)	

TDT	Transfusion dependent β -thalassemia
TIDE	Tracking of Indels by Decomposition
WT	Wild type

Related File(s)

IND # 18143; Autologous CRISPR-Cas9 Modified CD34+ Human Hematopoietic Stem and Progenitor Cells (CTX001), International Nonproprietary Name: exagamlogene autotemcel (exa-cel); Treatment of Transfusion Dependent Beta-Thalassemia (TDT) and Severe Sickle Cell Disease (SCD) in adult and pediatric patients; Vertex Pharmaceuticals Incorporated; ACTIVE

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INTRODUCTION

Hemoglobin (Hb) is a tetramer formed of 4 globin peptides, each tightly associated with a heme group that contains an atom of iron. During gestation, the predominant form of hemoglobin is HbF, which is composed of 2 α -globin chains and 2 γ -globin chains. Shortly before birth, there is a switch from HbF to adult hemoglobin (HbA), which contains 2 α -globin and 2 β -globin polypeptide chains. The switch from HbF to HbA is mediated by a transcriptional switch from γ -globin to β -globin within the β -globin gene cluster located on chromosome 11.

Hemoglobinopathies are disorders caused by genetic mutations that affect the production or function of adult hemoglobin (HbA) molecules. Patients with TDT and SCD share a common genetic etiology, both are caused by mutations in the β -globin gene included in HbA molecules.

Beta-thalassemia is an inherited autosomal recessive disorder caused by genetic mutations that reduce or eliminate the expression of β -globin, which results in an α - to non- α -globin chain imbalance and decrease in adult hemoglobin (HbA) tetramers in red blood cells (RBCs). Unpaired α -globin chains precipitate inside RBCs, leading to destruction of erythroid precursors in the bone marrow, known as ineffective erythropoiesis, and destruction of RBCs in circulation, known as peripheral hemolysis.¹ TDT is the most severe form of beta-thalassemia that is characterized by significant anemia, requiring regular (every 2 to 4 weeks) transfusions of RBCs for life, which carries risks for infection, iron overload resulting in end-organ damage, reduced quality of life and reduced survival.

CASGEVY consists of autologous CD34+ hHSPCs modified by CRISPR-Cas9-mediated genome editing technology of *BCL11A* gene at the GATA1 transcription factor-binding site, which is required for expression of *BCL11A* gene in erythroid cells. The reduction of *BCL11A* gene transcription and subsequent decrease in BCL11A protein leads to a switch from adult β -globin to a fetal γ -globin expression and resultant increase in HbF, which is similar to the phenotype of 'Hereditary Persistence of Fetal Hemoglobin', that has been documented in amelioration or absence of clinical disease manifestation in patients with SCD and beta-thalassemia.^{2, 3, 4, 5} Cas9 is an enzyme that cleaves a specific genomic locus that is complementary to the SPY101 gRNA. The intended mechanism of action is that repair of this Cas9 induced DSB by NHEJ can result in formation of indels that disrupt GATA1 binding in erythroid lineage cells, leading to decreased expression of the *BCL11A* gene, and thereby reactivating the expression of γ -globin mRNA in erythroid precursors, which in turn will lead to an increase in naturally occurring HbF protein levels in HSPCs that differentiate to erythroid cells.

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

¹ Thein SL. (2013) The molecular basis of beta-thalassemia. Cold Spring Harb Perspect Med. 3(5):a011700.

² Bauer DE, et al. (2012) Reawakening fetal hemoglobin: prospects for new therapies for the beta-globin disorders. Blood. 120(15):2945-53.

³ Lettre G & Bauer DE (2016) Fetal haemoglobin in sickle-cell disease: from genetic epidemiology to new therapeutic strategies. Lancet. 387(10037):2554-64.

⁴ Musallam KM, et al. (2013) Clinical experience with fetal hemoglobin induction therapy in patients with beta-thalassemia. Blood. 121(12):2199-212.

⁵ Thein SL (2005) Pathophysiology of beta thalassemia--a guide to molecular therapies. Hematology. 2005:31-7.

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of CASGEVY to treat the proposed clinical indication.

In Vitro Studies

Study Number	Study Title / Publication Citation	Report Number
1	Assessment of Functional Potential of the SPY101 RNP Edited Cells in Up-Regulation of γ -Globin Expression	CTxSR-010
2	Use of Single Cell-Derived Erythroid Colonies to Analyze Genotype-to-Phenotype Correlation of SPY101-Cas9 RNP-Edited CD34+ HSPCs from Healthy Donors	CTxSR-011
3	Functional Assessment of the Lead Guide RNA, SPY101, in Fetal Hemoglobin Upregulation in Primary Human CD34+ Cells from Healthy Donors	CTxSR-021
4	Characterization of GLP/Tox Lots – In vitro (b) (4) Assay	CTxSR-025
5	Characterization of GLP/Tox Lots – In vitro Erythroid Differentiation	CTxSR-026
6	Evaluation of SPY101 Editing in CD34+ Hematopoietic Stem/ Progenitor Cell Subpopulations in GLP/Tox Lots	CTxSR-023
7	Investigation of Editing Frequency, γ -Globin Transcript and HbF Upregulation Following Treatment of Sickle Cell Disease Patient Cells with SPY101-RNP	CTxSR-043
8	Investigation of Editing Frequency, γ -Globin Transcript and HbF Upregulation Following Treatment of Beta-Thalassemia Major Patient Cells with SPY101-RNP	CTxSR-031
9	Evaluation of SPY101 Editing in CD34+ Hematopoietic Stem and Progenitor Cell Subpopulations	CTxSR-012
10	Investigation of Editing Efficiency and γ -Globin Upregulation Potential of the Lead Guide RNA, SPY101, in Beta-Thalassemia Patient Cells	CTxSR-014
11	Evaluation of SPY101 Editing in CD34+ Hematopoietic Stem/Progenitor Cell Subpopulations in cGMP PQ lots	CTxSR-027
12	Characterization of CTX001 cGMP Process Quality (PQ) Lots (b) (4) – In vitro (b) (4) Assay	CTxSR-029
13	Characterization of cGMP PQ Lots (b) (4) – In vitro Erythroid Differentiation	CTxSR-030
14	Characterization of CTX001 cGMP Process Qualification (PQ) Lots (b) (4) – CD34+ Hematopoietic Stem and Progenitor Cell Subpopulation	CTxSR-035
15	Characterization of CTX001 cGMP Process Quality (PQ) Lots (b) (4) – In vitro (b) (4) Assay	CTxSR-037
16	Characterization of CTX001 cGMP PQ Lots (b) (4) – In vitro Erythroid Differentiation	CTxSR-038
17	Characterization of CTX001 cGMP Process Qualification (PQ) Lots (b) (4) – CD34+ Hematopoietic Stem and Progenitor Cell Subpopulation	CTxSR-039
18	Characterization of CTX001 GMP Process Qualification (PQ) Lots (b) (4) – In vitro (b) (4) Assay	CTxSR-041
19	Characterization of cGMP PQ Lots (b) (4) Derived from Plerixafor-only Mobilized Healthy Donor PBMCs – In vitro Erythroid Differentiation	CTxSR-042

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
20	An Engraftment Study of Genetically Modified and Non-modified CD34+ Cells Following a Single Intravenous Infusion in Irradiated NOD/SCID/IL2Rnull (NSG) Mice	2016-1955
20.1	Evaluation of Erythroid Differentiation Potential of Edited Human CD34+ Cells from Transplanted Mouse Bone Marrow by In Vitro Cell Culture	CTxSR-020
20.2	Characterization of Edits and Evaluation of Editing Persistence in a Xenotransplant Study of SPY101-RNP Treatment on Human Hematopoietic Stem Cells	CTxSR-033
21	Evaluation of Differentiation And Long-term Engraftment Potential of (b) (4) (SPY101)/Cas9 RNP-modified Evaluation of Differentiation and Long Term Engraftment Potential of (b) (4) (SPY101)/Cas9 RNP-Modified Human Mobilized Peripheral Blood CD34+ Hematopoietic Stem and Progenitor Cells After Transplantation Into Immune Compromised Mice	N199

Note: Due to the exploratory nature of the study and since CTX001 was not administered in the study, Study Report No. CTxSR-022 titled: ‘Assessment of Engraftment Potency in Cultured and Electroporated CD34+ Hematopoietic Stem and Progenitor Cells from Mobilized Peripheral Blood of Healthy Human Donors in Immunodeficient NSG Mice’ is not summarized in this review memo.

Note: CTX001 that was used in POC studies (Study Report Nos. CTxSR-010 and STxSR-011), was generated with spCas9 protein (b) (4) of Cas9 protein that was manufactured by (b) (4), while CTX001 that was used in the rest of the nonclinical studies was produced with spCas9 protein (b) (4) of the Cas9 protein that was manufactured by (b) (4). It was reported that (b) (4) Cas9 protein correlated with higher intracellular activity of Cas9-gRNA RNPs and higher on-target genome editing activity⁶, which is reflected in the submitted nonclinical study reports, with an average on-target gene editing frequency of 63.5(±11.3)% using spCas9 protein from (b) (4) (Study Report No. CTxSR-010) and average on-target gene editing frequencies of 77.9(±9.4)%, 75.7(±0.33)%, and 89.65(±3.6)% using the intended spCas9 protein from (b) (4) (Study Report Nos CTxSR-021, STxSR-023, CTx-SR-012). The on-target editing specificity of CTX001 in all nonclinical studies is expected to be the same due to the use of the SPY101 gRNA. Therefore, Study Report Nos CTxSR-010 and STxSR-011 were considered supportive of this BLA.

Overview of Pharmacology Studies

Overview of In Vitro Studies

Study #1

Study Report No: CTxSR-010

(b) (4)

Study Title: Assessment of Functional Potential of the SPY101 RNP Edited Cells in Up-Regulation of γ -Globin Expression

Objective: To assess the functional potential of the selected BCL11A/SPY101 gRNA by analyzing the γ -globin transcript and protein upregulation in mPB CD34+ hHSPCs edited by SPY101-RNP.

Study Agents:

- Test article: SPY101-RNP
- Negative control article: Enhanced green fluorescent protein (EGFP)-RNP with EGFP gRNA
- Positive control article: (b) (4)-RNP with (b) (4) gRNA
(Note: (b) (4))

Methods: CD34+ hHSPCs (1×10^5 cells/group) from four healthy donors ((b) (6)) were incubated with the Cas9-gRNA RNP complex ((b) (4) Cas9 (b) (4) and (b) (4) gRNA), followed by EP (b) (4). The genome edited cells were differentiated (b) (4) days towards the erythroid cell lineage followed by assessment for on-target editing and γ -globin expression.

- **On-target editing frequency (also named ‘efficiency’):** Determined by ‘Tracking of Indels by Decomposition’ (TIDE) analysis⁷ with PCR to amplify a 700 bp region surrounding the SPY101 gRNA cut site followed by Sanger sequencing on Days 1, 11, 13, and 15.
- **γ -globin expression:** γ -globin transcript levels were determined by (b) (4) on Days 11 and 15 of culture, and γ -globin protein levels were determined by (b) (4) on Day 15 of culture

Key Results:

- **On-target editing frequency:** SPY101-RNP edited CD34+ hHSPCs showed an average of $63.5 \pm 11.3\%$ on-target editing frequency from four healthy donors. The on-target editing frequencies remained stable to Day 15 of culture.
- **γ -globin expression:** SPY101-RNP-edited CD34+ hHSPCs demonstrated higher expression of γ -globin transcripts and γ -globin protein compared to the EGFP-RNP edited and unedited control hHSPCs at Days 11 and 15. However, SPY101-RNP edited CD34+ hHSPCs demonstrated around 50% (transcript and protein) expression of γ -globin compared to the positive control cells edited with (b) (4)-RNP that directly targets the BCL11A (b) (4).

Reviewer’s Conclusion:

⁷ Brinkman EK, et al. (2014) Easy quantitative assessment of genome editing by sequence trace decomposition. Nucleic Acid Res 42(22):e168 <https://doi.org/10.1093/nar/gku936>

- CASGEVY manufactured from healthy donors using spCas9 protein from (b) (4), demonstrated an average of 63.5(±11.3)% on-target gene editing frequency and upregulation of γ -globin transcript and protein to Day 15 in culture when differentiated toward an erythroid lineage.

Study #2

Study Report Number: CTxSR-011

Study Title: Use of Single Cell-Derived Erythroid Colonies to Analyze Genotype-to-Phenotype Correlation of SPY101-Cas9 RNP-Edited CD34+ HSPCs from Healthy Donors

Objective: To establish the relationship between the type of SPY101-RNP induced genetic modifications (genotype) to γ -globin expression (phenotype) in CD34+ hHSPCs.

Study Agents:

- Test article: SPY101-RNP
- Negative control article: EGFP-RNP

Methods: CD34+ hHSPCs (9.3×10^5 cells/group) from one healthy donor ((b) (6)) were incubated with the Cas9-gRNA RNP complex ((b) (4)) [(b) (4) and (b) (4)], followed by EP as described in Report No. CTxSR-010. (b) (4) post-EP, the edited cells underwent (b) (4) SPY101-RNP-edited colonies and (b) (4) control EGFP-RNP-edited colonies, were evaluated for correlation between genotype and phenotype

- Genotyping – The indels and disruption/deletion of the GATA1 binding site were determined by TIDE analysis. The indel size, location, and sequence of each allele was determined via (b) (4) software⁸
- Phenotyping for globin expression: α , β , γ -globin transcripts levels were determined by (b) (4) on Day 15

Key Results:

- Genotyping: Overall, 97% (318 of 328 alleles) of the allelic indels from 164 colonies had indels ≤ 50 bp in size surrounding the SPY101 gRNA cut-site. Three major identified indel species were detected: -15 (15 bp deletion; 18.9% of 318 alleles), +1 (1 bp insertion; 18.6% of 318 alleles) and -13 (13 bp deletion; 10.7% of 318 alleles). These three indel species were also observed with the highest frequency in bulk population of SPY101-RNP edited CD34+ hHSPCs before single cell sorting.

- Phenotyping: SPY101-RNP-edited colonies showed higher expression of γ -globin transcripts compared to the EGFP-RNP-edited negative control colonies. The α -globin and β -globin transcripts remained comparable between SPY101-RNP-edited colonies and EGFP-RNP-edited colonies.
- Genotype to phenotype correlation: Greater than 90% of the SPY101-RNP-edited colonies had either one allele with an on-target indel and one WT allele (mono-allelic) or both alleles with on-target indels (bi-allelic) causing disruption/deletion of the GATA1 binding site. 76% of the colonies were bi-allelic for on-target indels. There was a positive correlation between genotype of on-target indels alleles with disruption/deletion of GATA1 and upregulation of the γ -globin transcript. The colonies with bi-allelic GATA1 disruption/deletion showed significantly higher γ/α -globin and $\gamma/(\gamma+\beta)$ -globin (β -like globin) ratios compared to those with a mono-allelic genotype or without GATA1 binding site disruption/deletion.

Reviewer's Conclusion:

- CASGEVY manufactured from healthy donors using spCas9 protein from (b) (4) demonstrated three major indel species: -15 (15 bp deletion), -13 (13 bp deletion) and +1 (1 bp insertion). There was a positive correlation between genotypes of on-target indels leading to GATA1 disruption/deletion and the upregulated γ -globin phenotype.

Study #3

Study Report No.: CTxSR-021

Study Report Title: Functional Assessment of the Lead Guide RNA, SPY101, in Fetal Hemoglobin Upregulation in Primary Human CD34+ Cells from Healthy Donors

Objective: To assess the functional potential of SPY101-RNP in upregulating the γ -globin protein as HbF using (b) (4) in SPY101-RNP-edited CD34+ hHSPCs originated from mobilized peripheral blood and differentiated to the erythroid lineage.

Study Agents:

- Test article: SPY101-RNP
- Negative control article: EGFP-RNP
- Positive control article: (b) (4)-RNP

Methods: CD34+ hHSPCs (1×10^5 cells/group) from three healthy donors ((b) (6)) were incubated with the Cas9-gRNA RNP complex ((b) (4) Cas9 [(b) (4)] and (b) (4)), followed by EP as described in Report No. CTxSR-010, followed by differentiation towards the erythroid lineage. The edited cells were evaluated by the following analyses:

- On-target editing frequency was determined by TIDE analysis on Days 0, 11, and 13.
- γ -globin transcript levels were determined by (b) (4) on Days 13 and 15.
- Hemoglobin (HbF and HbA) levels were determined by (b) (4) on Days 13, 15, 18, and 21.

Key Results:

- On-target editing frequency: SPY101-RNP-edited CD34+ hHSPCs showed stable editing frequencies (74.7 (\pm 13.1)% to 77.9 (\pm 9.4)%) throughout erythroid differentiation.
- γ -globin transcript levels: SPY101-RNP-edited CD34+ hHSPCs showed upregulation of γ -globin gene (as indicated by increased γ/α -globin and $\gamma/(\beta+\gamma)$ -globin ratios) compared to EGFP-RNP- edited CD34+ hHSPCs on Days 13 and 15. The upregulation of the γ -globin gene in SPY101-RNP edited CD34+ hHSPCs was ~50% lower compared to (b) (4)-RNP-edited CD34+ hHSPCs on Days 13 and 15.
- HbF level: SPY101-RNP-edited CD34+ hHSPCs showed upregulation of HbF protein levels (as indicated by increased HbF/HbA and HbF/(HbF + HbA) ratios) compared to EGFP-RNP- edited CD34+ hHSPCs on Days 13, 15, 18, and 21.

Reviewer's Conclusion:

- CTX001 manufactured from healthy donors demonstrated stable on-target editing frequencies (74.7 (\pm 13.1)% to 77.9 (\pm 9.4)%) during erythroid differentiation. Editing with the SPY101-RNP mediated upregulation of the γ -globin transcript and HbF protein levels.

Study #4

Study Report No.: CTxSR-025

Study Report Title: Characterization of GLP/Tox Lots – *In vitro* (b) (4) Assay

Objective: To assess the multi-lineage potential of nonclinical lots of CTX-001.

Study Agents:

- Test article: CTX001 manufactured using the same procedure as the commercial manufacturing process from healthy donors (b) (6)
- Control article: Unedited CD34+ hHSPCs from each donor

Methods: (b) (4)

Key Results:

- CASGEVY and unedited CD34+ hHSPCs showed comparable (b) (4) distribution of (b) (4).

Reviewer's Conclusion:

- SPY101-RNP editing of the CD34+ hHSPCs from healthy donors did not significantly affect the (b) (4) distribution profile of erythroid and myeloid progenitor (b) (4).

Study #5

Study Report No.: CTxSR-026

Study Report Title: Characterization of GLP/Tox Lots – *In vitro* Erythroid Differentiation

Objective: To assess whether: 1) nonclinical lots of CASGEVY can differentiate into the erythroid lineage *in vitro* and 2) if the differentiated cells express elevated levels of γ -globin transcripts compared to unedited CD34+ hHSPCs.

Study Agents:

- Test article: CTX001 manufactured using the same procedure as the commercial manufacturing process from three healthy donors ((b) (6))
- Control article: Unedited CD34+ hHSPCs from each donor

Methods: CTX001 and unedited cells from each donor were thawed and differentiated towards the erythroid lineage for 21 days, and then evaluated by the following analyses:

- Cell viability and cell growth were determined by (b) (4) every other day from Days 3 to 21.
- Expression of erythroid surface markers CD71 (a surface marker of erythroid lineage), GlyA (also named CD235, expressed in erythrocytes and erythroid precursors), $\alpha 4$ -integrin (a subunit of $\alpha 4 \beta 1$ expressed in various immune cells, e.g., T-, B-, progenitor cells, nature killer cells, etc.), Band 3 (also named AE1, an anion transport protein/important structural component of erythrocyte cell membrane) were determined by FACS analysis on Days 7, 10, 12, 14, and 15.
- Enucleation analysis: Conducted on Days 10, 12, 14, and 18
- γ -globin transcript levels determined by (b) (4) on Day 13
- Hemoglobin (HbF and FbA) protein levels determined by (b) (4) on Day 18

Key Results:

- CTX001 compared to unedited CD34+ hHSPCs showed 1) similar cell viability and cell growth during 21-days of culture for erythroid differentiation, 2) similar expression of erythroid surface markers CD71+, GlyA+, $\alpha 4$ -Integrin+, or Band 3+ on Days 7, 10, 12, 14 and 18 of erythroid differentiation, 3) similar enucleation at Days 10, 12, 14, and 18 of erythroid differentiation, 4) significantly higher ($p=0.014$) $\gamma/(\gamma+\beta)$ (~1.93x) expression at Day 13 of erythroid differentiation in CTX001 compared to unedited control CD34+ hHSPCs, and 5) significantly higher ($p=0.0004$) HbF/(HbF+HbA) (~2.3x)

expression at Day 18 of erythroid differentiation in CTX001 compared to unedited control CD34+ hHSPCs.

Reviewer's Conclusion:

- SPY101-RNP mediated genome editing did not significantly affect cell viability, cell growth, erythroid differentiation, including expression of erythroid surface markers, and enucleation. SPY101-RNP editing resulted in upregulation of *γ-globin* transcript and HbF protein in CD34+ hHSPCs from healthy donors.

Study #6

Study Report Number: CTxSR-023

Study Title: Evaluation of SPY101 Editing in CD34+ Hematopoietic Stem/Progenitor Cell Subpopulations in GLP/Tox Lots

Objective: To assess CTX001 derived from healthy donors) for: 1) viability, 2) proportion of subpopulations of CD34+ hHSPCs, and 3) on-target editing frequency.

Study Agents:

- Test article: CASGEVY manufactured by the intended procedure at the intended manufacturing site ((b) (4))
- Control article: Unedited CD34+ hHSPCs from each donor

Methods: CTX001 from three healthy donors ((b) (6)) and unedited cells from each donor were thawed and underwent cell differentiation toward an erythroid lineage for 21 days, and were evaluated by the following analyses:

- Cell viability determined by (b) (4)
- Frequency of hHSPCs progenitor subpopulations determined by Flow Cytometry/ FACS including LT-HSC, MPP, MLP, GMP, CMP/MEP subpopulations were sorted.
- On-target editing frequency of CTX001 bulk (unsorted) and various sorted lineages of progenitor subpopulations was determined by TIDE analysis as described in Report No. CTxSR-011.

Key Results:

- Compared to unedited cells, CTX001 showed similar cell viability (87-90%) and similar distribution profile of various lineage progenitor subpopulations (Table #1). The on-target editing frequencies are similar in bulk (unsorted) and sorted lineages of progenitor subpopulations (Table #2).

Table #1: Sub-population Frequencies (%)

Subpopulation	Unedited CD34+ HSPCs	SPY101-RNP-edited CD34+ HSPCs
CD34+	96.45 ± 1.34 %	97.70 ± 0.85 %

LT-HSC	9 ± 6.8 %	9.71 ± 8.47 %
MPP	13.45 ± 4.74 %	9.72 ± 4.50 %
MLP	2.06 ± 1.84 %	2.11 ± 2.07 %
GMP	8.17 ± 4.86 %	8.36 ± 2.89 %
CMP/MEP	16.05 ± 8.13 %	13.75 ± 4.30 %

Source – page 13 of ctxsr-023.pdf in Module 4.2.1.1 ‘Primary Pharmacodynamics’

Table #2: SPY101-RNP On-Target Editing Frequency (%) in CD34+ hHSPCs and subpopulation

Subpopulation	SPY101-RNP-edited CD34+ HSPCs
Bulk	75.65 ± 2.33 %
LT-HSC	77.5 ± 1.27 %
MPP	80.2 ± 0.57 %
MLP	83.15 ± 5.45 %
GMP	83.8 ± 1.84 %
CMP/MEP	84.6 ± 1.27 %

Source – page 13 of ctxsr-023.pdf in Module 4.2.1.1 ‘Primary Pharmacodynamics’

Reviewer’s Conclusion:

- SPY101-RNP-editing did not significantly impact the cell viability or progenitor subpopulation distribution profile in CD34+ hHSPCs from healthy donors. The on-target editing efficiency (76-85%) was similar in bulk unsorted CASGEVY and the sorted progenitor subpopulations.

Study #7

Study Report No.: CTxSR-043

Study Report Title: Investigation of Editing Frequency, γ -Globin Transcript and HbF Upregulation Following Treatment of Sickle Cell Disease Patient Cells with SPY101-RNP

Objective: To assess the editing frequency of SPY101-RNP-edited CD34+ hHSPCs from patients with SCD.

Study Agents:

- Test article: SPY101-RNP
- Negative control article: EGFP-RNP

Methods: CD34+ hHSPCs (1-2 x10⁵ cells/group) from ten patients with SCD (b) (6)

(b) (6) and four healthy donors (b) (6) were incubated with the Cas9-gRNA RNP complex (b) (4) Cas9 [(b) (4)] and (b) (4) gRNA), followed by EP same as described in Report No. CTxSR-010 and cell differentiation toward erythroid lineage. The cells were evaluated by the following

analyses:

- On-target editing frequency and indel distribution determined by TIDE analysis
- γ -globin transcript expression determined by (b) (4)
- HbF levels determined by (b) (4)

Key Results:

- On-target editing frequency (%Indels): CTX001 manufactured from healthy donors and patients with SCD showed similar but variable on-target editing frequencies ranging from 53.3% to 80% in CTX001 manufactured from healthy donors and 42.4% to 88% in CASGEVY manufactured from patients with SCD.
- γ -globin transcript and HbF protein levels: CTX001 from healthy donors and patients with SCD showed similar but highly variable results including upregulation γ -globin, reported as higher $\gamma/(\gamma+\beta)$ -globin, ranging from ~2.9X-10.3X in CTX001 manufactured from healthy donors, and ~1.3X-7.6X in CTX001 manufactured from patients with SCD, upregulation of HbF, reported as higher HbF/total Hb, ranging from ~3.6X-6.6X in CTX001 manufactured from healthy donors and ~2.4X- 4.2X in CTX001 manufactured from patients with SCD compared to EGFP-RNP edited control cells.

Reviewer's Conclusion:

- SPY101-RNP edited CD34+ hHSPCs from healthy donors and patients with SCD showed similar on-target editing frequency, upregulation of γ -globin transcript, and HbF protein compared to the EGFP-RNP edited control cells. The observed variability in the results could be due to donor-to-donor variation.

Study #8

Study Report No.: CTxSR-031

Study Report Title: Investigation of Editing Frequency, γ -Globin Transcript and HbF Upregulation Following Treatment of Beta-Thalassemia Major Patient Cells with SPY101-RNP

Objective: To assess SPY101-RNP-editing frequency with CD34+ hHSPCs obtained from patients with β -thalassemia.

Study Agents:

- Test article: SPY101-RNP
- Negative control article: EGFP-RNP

Methods: CD34+ hHSPCs (1.5×10^5 cells/group) from three patients with beta-thalassemia (b) (6) and one healthy donor (b) (6) were incubated with the Cas9-gRNA RNP complex ((b) (4) Cas9 [(b) (4)] and (b) (4) gRNA), followed by EP as described in Report No.

CTxSR-010 and cell differentiation towards the erythroid lineage. The cells were evaluated by the following analyses:

- On-target editing frequency and indel distribution were determined by TIDE analysis on Day 9
- γ -globin transcript expression was determined by (b) (4) on Day 17
- HbF levels based on γ -globin protein expression were determined by (b) (4) on Day 18 or 19

Key Results:

- On-target editing frequency and indel distribution: CTX001 manufactured from a healthy donor and patients with beta-thalassemia demonstrated similar editing frequencies (%Indels) of 86% to 91% and indel distribution profiles of on-target editing including 15 bp deletion at 18.5 to 24.2%, 13 bp deletion at 17% to 23.2%, and 1 bp insertion at 28.3% to 35.1%.
- γ -globin transcript and HbF levels: CTX001 from healthy donors or patients with beta-thalassemia showed upregulation of γ -globin expression, reported as higher γ/α -globin, ranging from ~5X-7.3X in CTX001 manufactured from a healthy donor and ~2X-5X in CTX001 manufactured from patients with beta-thalassemia, and upregulation of HbF, reported as higher HbF/(HbF+HbA), ranging from ~3X-4.4X in CTX001 manufactured from a healthy donor and ~1.3X-3X in CTX001 manufactured from patients with beta-thalassemia compared to unedited control cells.

Reviewer's Conclusion:

- CTX001 that was manufactured from a healthy donor and three patients with beta-thalassemia showed similar on-target editing frequencies of 86% to 91%, similar indel distribution profiles, and upregulation of γ -globin transcript expression and HbF protein compared to the unedited control cells.

Study #9

Study Report No.: CTxSR-012

Study Report Title: Evaluation of SPY101 Editing in CD34+ Hematopoietic Stem and Progenitor Cell Subpopulations

Objective: To determine on-target editing frequencies in subpopulations of SPY101-RNP edited cells

Study Agent:

- Test article: SPY-101-RNP

Method: CD34+ hHSPCs (0.25×10^6 , 1×10^6 , or 20×10^6 cells/group) from four healthy donors ((b) (6)) were incubated with the SPY-101 RNP complex ((b) (4))

(b) (4) Cas9 [(b) (4)] and (b) (4) gRNA) followed by EP using the cell transfection apparatus MaxCyte. The cells were cultured for additional (b) (4) after EP, and sorted for MPP, MLP, GMP, CMP/MEP subpopulations by FACS analysis. The on-target editing frequencies were determined from genome edited cells before and after FACS including bulk unsorted and MPP, MLP, GMP, and CMP/MEP sorted subpopulations by TIDE analysis.

Key Results:

- CTX001 manufactured from healthy donors showed similar on-target editing frequencies (88% to 92%) in bulk unsorted cells and various lineage progenitor subpopulations.

Reviewer's Conclusion:

- CTX001 manufactured from healthy donors using the MaxCyte system showed on-target editing frequencies of 89.65(±3.6)% in bulk unsorted cells and in various lineage progenitor subpopulations (MPP, MLP, GMP, and CMP/MEP).

Study #10

Study Report No.: CTxSR-014

Study Report Title: Investigation of Editing Efficiency and γ -Globin Upregulation Potential of the Lead Guide RNA, SPY101, in Beta-Thalassemia Patient Cells

Objective: To assess SPY101-RNP-editing frequency with CD34+ hHSPCs obtained from patients with beta-thalassemia.

Donor cell lines:

- CD34+ hHSPCs from two patients with beta-thalassemia: (b) (6) and ID (b) (6)
- CD34+ hHSPCs from healthy donors: (b) (6)

Study Agents:

- Test article: SPY101-RNP
- Negative control article: EGFP-RNP

Method: CD34+ hHSPCs (2 x 10⁵ cells/group) from two patients with beta-thalassemia (b) (6) and ID (b) (6) and two healthy donors ((b) (6)) were incubated with the Cas9-gRNA RNP complex ((b) (4) Cas9 [(b) (4)] and (b) (4) gRNA), followed by EP as described in Report No. CTxSR-010 and cells were differentiated towards the erythroid lineage. The cells were evaluated by the following analyses:

- On-target editing frequency and indel distribution determined by TIDE analysis 7 days post-EP
- γ -globin transcript expression levels determined by (b) (4) on Day 13

- γ -globin protein expression levels determined by flow cytometry Day 15

Key Results:

- On-target editing frequency and indel distribution: Experiments 1 and 2 showed similar results. CTX001 manufactured from patients with beta-thalassemia showed a higher percentage of indels (84% to 85%) compared to CTX001 manufactured from healthy donors (53% to 58%); similar indel distribution profiles were observed between the patient samples and the healthy donors including 15 bp deletion (10% in healthy donors, 21% in patients with beta-thalassemia), 13 bp deletion (11% in healthy donors and 20% in patients with beta-thalassemia) and 1 bp insertion (20% to 23% in healthy donors and 30% to 36% in patients with beta-thalassemia).
- γ -globin transcript expression and protein expression: CTX001 manufactured from patients with beta-thalassemia and healthy donors showed upregulation of γ -globin transcript expression, reported as higher $\gamma/(\gamma+\beta)$ -globin ratios, ranging from ~3X-6X from CTX001 manufactured from healthy donors and ~2X-2.3X from CTX001 manufactured from patients with beta-thalassemia, and upregulation of γ -globin protein expression of ~4.7X from CTX001 manufactured from a healthy donor and ~2.8X from CTX001 manufactured from a patient with beta-thalassemia compared to EGFP-RNP edited control cells.

Reviewer's Conclusion:

- CTX001 manufactured from patients with beta-thalassemia showed a higher frequency of on-target editing and a similar indel distribution and upregulation of γ -globin transcript and γ -globin protein expression compared to healthy donors. It cannot be determined if the difference in on-target editing frequencies between healthy donors and patients with beta-thalassemia patients is due to donor-to-donor variation because of the limited number of analyzed samples.

Supporting Studies of GMP materials for CASGEVY

The following studies were conducted for characterization of the GMP materials of CASGEVY manufactured at (b) (4).

- **Study #11** (Report No. CTxSR-027): Three cGMP Process Qualification (PQ) lots that were manufactured from the (b) (4) G-CSF mPB of three healthy donors ((b) (6)), showed no SPY101-RNP editing-related changes for cell viability or the profile of CD34+ cell lineages subpopulations for LT-HST, MMP, MLP, GMP, and CMP/MEP. Similar SPY101-RNP related on-target editing frequencies (76 to 86%) were observed in bulk, and FACS-sorted lineage progenitor subpopulations: LT-HST, MMP, MLP, GMP, and CMP/MEP.
- **Study #12** (Report No. CTxSR-029): Three cGMP PQ lots from three healthy donors ((b) (4)) showed no SPY101-RNP editing-related changes on

differentiation frequencies of myeloid progenitor ((b) (4)) and erythroid progenitor ((b) (4)).

- **Study #13** (Report No. CTxSR-030): Three cGMP PQ lots that were manufactured from G-CSF mPB of three healthy donors ((b) (6)) showed i) no SPY101-RNP editing related changes on erythroid cell differentiation (%CD71+, %GlyA+, %α4-Integrin+, and %Band 3+ cells) during Days 7 to 18 of erythroid differentiation, or enucleation (%enucleation) during Days 10 to 18 of erythroid differentiation, and ii) SPY101-RNP editing related upregulation of γ-globin transcript (higher γ/α -globin and higher γ/(γ+β)-globin) and HbF (higher HbF/(HbF+HbA) at Days 13 and 18 of erythroid differentiation, respectively.
- **Study #14** (Report No. CTxSR-035): Four cGMP PQ lots ((b) (4)) that were manufactured from G-CSF and plerixafor mPB of two healthy donors showed: i) no SPY101-RNP editing related changes on distribution profile of various lineages of progenitors subpopulations (MPP, MLP, GMP, and CMP/MEP) and ii) similar on-target editing frequencies (76 to 85% indel) in bulk unsorted, and various lineages of progenitor subpopulations MPP, MLP, GMP, and CMP/MEP.
- **Study #15** (Report No. CTxSR-037): Two cGMP PQ lots ((b) (4)) that were manufactured from G-CSF and plerixafor mPB of two healthy donors, showed no SPY101-RNP editing related changes on differentiation to myeloid progenitor cells and erythroid progenitor cells by ((b) (4)) assay
- **Study #16** (Report No. CTxSR-038): Two cGMP PQ lots ((b) (4)) that were manufactured from G-CSF and plerixafor mPB of two healthy donors showed no SPY101-RNP editing related changes on erythroid cell differentiation (based on %CD71+, %GlyA+, %α4-Integrin+, and %Band 3+ cells) during Days 7-18 and enucleation at Days 14, and 18 of erythroid differentiation; and upregulation of γ-globin at Day 13 and HbF at Day 18 of erythroid differentiation.
- **Study #17** (Report No. CTxSR-039): Two cGMP PQ lots ((b) (4)) that were manufactured from the ((b) (4)) (plerixafor) mPB of two healthy donors, showed no SPY101-RNP editing related changes on profiles of various lineages of progenitor subpopulations (%LT-HSC, %MPP, %MLP, %GMP, and %CMP/MEP); and similar on-target editing frequencies (80 to 90%) in bulk and various lineage of progenitor subpopulations LT-HSC, MPP, MLP, GMP, and CMP/MEP.
- **Study #18** (Report No. CTxSR-041): Two cGMP PQ lots ((b) (4)) that were manufactured from two healthy donors showed no SPY101-RNP editing related changes on differentiation to myeloid progenitor cells and erythroid progenitor cells
- **Study #19** (Report No. CTxSR-042): Two cGMP PQ lots ((b) (4)) that were manufactured from two healthy donors showed no SPY101-RNP

editing related changes on erythroid cell differentiation during Days 7 to 14 of erythroid differentiation and enucleation (%enucleation) during Days 10 to 14 of erythroid differentiation; and upregulation of γ -globin at Day 13 and HbF at Day 18 of erythroid differentiation.

Overview of In Vivo Studies

Note:

- No relevant animal model of beta-thalassemia are available for POC studies for CASGEVY because the SPY101 gRNA is species-specific for the human gene sequence and there is no corresponding sequence in the murine genome. Therefore, no *in vivo* POC studies were performed in animal model of beta-thalassemia.

Study #20

Report Number		2016-1955
Date Report Signed		02/27/2018
Title		An engraftment study of genetically modified and non-modified CD34+ cells following a single intravenous infusion in irradiated NOD/SCID/IL2R γ null (NSG) mice
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To assess and compare engraftment of genetically edited and unedited CD34+ cells via a single IV injection in NSG mice
Study Animals	Strain/Breed	NOD (b) (4) ^{scid} IL2rg (b) (4) (NSG)
	Species	Mice
	Age	6-8 weeks old
	Body Weight	17-27 g
	#/sex/group	10 to 16 females/group for CD34+ cells obtained from each donor
	Total #	270
Conditioning		All study mice received conditioning by total body irradiation at 160 cGy/min for 200 cGy/mouse on Day 0
CD34+ hHSPCs from healthy donors		Donors #1 ((b) (6)), Donor #2 ((b) (6)), and Donor #3 ((b) (6)) Note: Groups 1-5 contain three donor cohorts. Each donor cohort evaluated control and test articles that were generated from the same donor (as listed below).
Test Article(s)		<ul style="list-style-type: none"> CTX001 – SPY101-RNP edited CD34+ from each donor

Control Article(s)	<ul style="list-style-type: none"> • Unedited CD34+ cells from each donor • Mock EP CD34+ cells without CRISPR/Cas9 editing components from each donor • GFP-RNP edited CD34+ cells from each donor • (b) (4)-RNP edited CD34+ cells from each donor <p>(b) (4) gRNA targets (b) (4)</p> <p>Note: Per the applicant, multiple potential candidate gRNAs were assessed including SPY101 and (b) (4) during nonclinical development. Results from evaluation of both gRNAs were reported in Study Reports Nos 2016-1955, 1016-2475, and 1016-2465. SPY101 was selected and moved into clinical development.</p>
Route of Administration	IV (Day 0)
Study Groups and Dose Levels	<p>Group 1 – Unedited CD34+ cells</p> <p>Group 2 – Mock EP CD34+ cells</p> <p>Group 3 – GFP-RNP edited CD34+ cells</p> <p>Group 4 – (b) (4)-RNP edited CD34+ cells</p> <p>Group 5 – CASGEVY (SPY101-RNP edited CD34+ cells)</p> <p>Group 6 – No injected cells</p>
Dosing Regimen	Single administration
Randomization	Yes (based on BWs)
Description of Masking	Not described
Scheduled Sacrifice Time Points	Week 16

Key Evaluations and Assessments:

- Engraftment of human (hCD45RA+) cells in whole blood at Weeks 8 and 16, in bone marrow at Week 16, and spleen at Week 16.
- Multilineage differentiation of transplanted CD34+ hHSPCs to B cells (CD19+), T cells (CD3+), and myeloid cells (CD33+) in whole blood, bone marrow, and spleen at Week 16.

Key Results:

- Single IV administration of CTX001 or unedited CD34+ hHSPCs in total body irradiated NSG mice resulted in:
 - Similar chimerism of transplanted cells in blood (32%- 42% at Week 8 and 4%-12.9% at Week 16), bone marrow (46%-65% at Week 16), and spleen (27%-50% at Week 16)
 - Similar multilineage differentiation of transplanted cells to B cells, T cells, and myeloid cells, in whole blood, bone marrow, and spleen at Week 16.
 - The level of chimerism of transplanted cells in blood was higher at Week 8 compared to Week 16.

Reviewer's Conclusion:

- Single IV administration of CTX001 at 1×10^6 cells/mouse in irradiated NSG mice resulted in no consistent patterns of SPY101-RNP editing related changes in chimerism or

multilineage cell differentiation to B-, T-, and myeloid cells in whole blood, bone marrow, and spleen compared to unedited CD34+ hHSPCs.

Study #20.1

Note: This study is an analysis of samples from study report number 2016-1955.

Study Report Number: CTxSR-020

Study Report Title: Evaluation of Erythroid Differentiation Potential of Edited Human CD34+ Cells from Transplanted Mouse Bone Marrow by *In Vitro* Cell Culture

Objective: To determine whether SPY101-RNP editing affects erythroid differentiation and maintains the same degree of on-target editing in bone marrow 16 weeks after transplantation in NSG mice.

Testing Facility: CRISPR Therapeutics

(b) (4)

Samples: The bone marrow samples were obtained from the following Study Groups of Study Report No. 2016-1955 at 16 weeks post IV administration:

- Group 3 – EGFP-RNP edited CD34+ hHSPCs
- Group 5 – CTX001

Assessment methods:

- (b) (4) assay: Erythroid progenitor lineage ((b) (4))
- On-target editing frequency: Indels from (b) (4) human (b) (4) by TIDE analysis

Key Results:

- The bone marrow harvested at 16-weeks post transplantation of CTX001 showed i) similar erythroid progenitor lineage ((b) (4)) output compared to control EGFP-RNP edited CD34+ cells; and ii) increased average SPY101-RNP on-target editing frequency ($93.8 \pm 8.477\%$) compared to control EGFP edited CD34+ hHSPCs ($2.62 \pm 2.04\%$).

Reviewer's Conclusion:

- CTX001 administered to mice showed an average of $93.8 \pm 8.477\%$ on-target editing frequency in erythroid progenitor cells in bone marrow 16 weeks post-administration.

Study #20.2

Note: This study is an analysis of samples from study report number 2016-1955.

Study Report Number: CTxSR-033

Study Report Title: Characterization of Edits and Evaluation of Editing Persistence in a Xenotransplant Study of SPY101-RNP Treatment on Human Hematopoietic Stem Cells

Objective: To evaluate CTX001 by comparing the on-target editing of the cells pre-transplantation and 16 weeks post-transplantation in NSG mice.

Testing Facility:

- Amplicon NGS:
(b) (4)
- Amplicon Indel Detection (AIDE) Analysis:
CRISPR Therapeutics
(b) (4)

Samples: The blood, bone marrow, and spleen samples were obtained from the following Study Group of Study Report No. 2016-1955 at 16 weeks post IV administration of CTX001:

- Group 5 – CTX001

Assessment methods:

- Amplicon NGS: On-target amplification and next-generation sequencing of the on-target region
- On-target editing frequency: On-target editing frequencies of various indel species by Amplicon Indel Detection (AIDE) software^{9, 10, 11}

Key Results:

- At 16 weeks post-transplantation, the blood, bone marrow, and spleen of CASGEVY-administered mice had on-target indel frequencies of 94.6±5.0%, 91.4±15.1%, and 96.8±1.7%, respectively. The most prevalent indel was a single T nucleotide insertion (+1) at the cut site. On-target editing frequencies at the GATA1 site were 85.5±6.0%, 82.3±14.2%, and 87.3±1.6% in blood, bone marrow, and spleen, respectively at 16-weeks post transplantation.

Reviewer's Conclusion:

- At 16 weeks post-transplantation, CASGEVY-administered mice showed similar on-target editing frequencies and a similar frequency of disruption of the GATA1 site in blood, bone marrow, and spleen.

⁹ Sheely A & Kernysky A (2017) Bioinformatics pipeline software design specification. CRISPR Therapeutics, VAL-IT-009 Cambridge MA

¹⁰ Sheely A & Kernysky A (2017) Bioinformatics pipeline Test Script. CRISPR Therapeutics, VAL-IT-025 Cambridge MA

¹¹ Sheely A & Kernysky A (2017) Bioinformatics pipeline System Release Memo. CRISPR Therapeutics, VAL-IT-026 Cambridge MA

Study #21

Report Number		N199
Date Report Signed		No signature page was included
Title		Evaluation of differentiation and long-term engraftment potential of (b) (4) (SPY101)/Cas9 RNP-modified human mobilized peripheral blood CD34+ hematopoietic stem and progenitor cells after transplantation into immune-compromised mice
GLP Status		No
Testing Facility		Vertex
Objective(s)		To test the engraftment potential of genetically modified human (CD34+) HSPCs in immune-compromised NSG mice.
Study Animals	Strain/Breed	NOD. (b) (4) ^{scid} Il2rg ^{-/-} (b) (4) (NSG)
	Species	Mice
	Age	4-6 weeks old
	Body Weight	Not specified
	#/sex/group	20 female mice/group
	Total #	60
Conditioning		All study mice were gamma-irradiated at 2 Gy/mouse at Day 0
CD34+ hHSPCs		Mobilized peripheral blood CD34+ hHSPCs ((b) (4)), Lot# (b) (4) purchased from (b) (4)
Test Article(s)		CTX001 (SPY101-RNP edited CD34+ hHSPCs) – CD34+ hHSPCs from healthy donors ((b) (4)) were treated with SPY101-RNP ((b) (4) [SPY101] sgRNA [(b) (4)] mixed with (b) (4) Cas9 protein [(b) (4)] followed by EP using MaxCyte GT transfection system [MaxCyte]).
Control Article(s)		<ul style="list-style-type: none"> No EP/unedited human CD34+ cells EP mock (without SPY101-RNP) human CD34+ cells
Route of Administration		IV (tail vein) on Day 0
Study Groups and Dose Levels		Group 1 (n=20 mice/group) – No EP CD34+ cells (5 x 10 ⁵ cells/mouse) Group 2 (n=20 mice/group) – Mock EP CD34+ cells (5 x 10 ⁵ cells/mouse) Group 3 (n=20 mice/group) – CTX001 (5 x 10 ⁵ cells/mouse)
Dosing Regimen		Single administration
Randomization		Not described
Description of Masking		Not described
Scheduled Sacrifice Time Points		20 weeks post-administration

Key Evaluations and Assessments:

- Engraftment of hCD45RA+ cells in whole blood at 8-, 12-, 16-, and 20-weeks post-administration.
- Engraftment of hCD45RA+ cells in bone marrow and spleen at 20 weeks post-administration.
- Multilineage differentiation of transplanted CD34+ hHSPCs to B- (CD19+), T- (CD3+), and myeloid (CD33+) cells in whole blood, bone marrow, and spleen at 20 weeks post-administration.

Key Results:

- Single IV administration of CTX001 or control CD34+ hHSPCs in γ -irradiated NSG mice, resulted in i) similar engraftment in whole blood at 8-, 12-, 16- and 20-weeks post-administration, ii) similar engraftment in bone marrow and spleen at 20-week post-

administration, and ii) similar multilineage differentiation of transplanted cells to B-, T-, and myeloid cells in whole blood, bone marrow, and spleen at 20-weeks post-administration.

Reviewer's Conclusion:

- Single IV administration of CASGEVY at 1×10^6 cells/mouse in total-body irradiated NSG mice, resulted in no consistent patterns of SPY101-RNP editing related changes in engraftment or differentiation in whole blood, bone marrow, and spleen up to 20 weeks post-administration.

SAFETY PHARMACOLOGY STUDIES

There were no safety pharmacology studies conducted with CASGEVY.

PHARMACOKINETIC STUDIES

Summary List of Pharmacokinetics Studies

The following biodistribution (BD) study was conducted with CASGEVY.

Study Number	Study Title / Publication Citation	Report Number
22	A Pivotal Biodistribution and Persistence Study of Genetically Edited and Non-Edited CD34+ Cells Following a Single Intravenous Injection to Irradiated NOD/SCIDIL2R γ null (NSG) Mice	1016-2475

Overview of BD Study

Study #22

Report Number	1016-2475
Date Report Signed	02/12/2018
Title	A Pivotal Biodistribution and Persistence Study of Genetically Edited and Non-Edited CD34+ Cells Following a Single Intravenous Injection to Irradiated NOD/SCID/IL2R γ null (NSG) Mice
GLP Status	<p>Yes</p> <p>The study conduct complied with OECD principles of GLP, except for the following:</p> <ul style="list-style-type: none"> • The (b) (4) analysis at (b) (4) • Immunohistochemistry (IHC) at (b) (4) • (b) (4) and amplicon NGS analyses • Statistical analysis on (b) (4) analysis results • NGS Amplicon analysis (performed by CRISPR Therapeutics)
Testing Facility	(b) (4)
Objective(s)	To assess and compare engraftment, biodistribution, and persistence of locus specific CRISPR/Cas9 genetically edited and non-edited CD34+ hHSPCs.

Study Animals	Strain/Breed	NOD: (b) (4) ^{scid} Il2rg (b) (4) (NSG)																						
	Species	Mice																						
	Age	7-9 weeks old																						
	Body Weight	23-31 g (males); 18-25 g (females)																						
	#/sex/group	15 sex/group/sacrifice time point for Groups 1-3, 3/sex/group/sacrifice time point for Group 4																						
	Total #	192																						
Conditioning	All study mice received conditioning of total body irradiation at 160 cGy/min for 200 cGy/mouse on Day 0																							
CD34+ hHSPCs from healthy donors	Donors #1 ((b) (6)), Donor #2 ((b) (6)), and Donor #3 ((b) (6)) (provided by (b) (4)) Note: Groups 1 to 3 contains three donor cohorts and each donor cohort evaluated control and test articles that were generated from the same donor (as listed below) .																							
Test Article(s)	<ul style="list-style-type: none">CTX001 (SPY101-RNP edited CD34+ hHSPCs) from each donor																							
Control Article(s)	<ul style="list-style-type: none">(b) (4)-RNP edited CD34+ hHSPCs from each donorUnedited CD34+ hHSPCs from each donorInjection buffer (PBS, 0.1% human serum albumin) * The (b) (4) gRNA is the same as in Report No. 2016-1955 that (b) (4) .																							
Route of Administration	IV on Day 0																							
Study Groups and Dose Levels	<table><tr><th>Group</th><th>Study Agent</th><th>Dose Level (cells/mouse)</th><th>N/Sex/Group/Sacrificed time point</th></tr><tr><td>1</td><td>Unedited CD34+ hHSPCs</td><td>1 x 10⁶</td><td>15</td></tr><tr><td>2</td><td>CASGEVY</td><td>1 x 10⁶</td><td>15</td></tr><tr><td>3</td><td>(b) (4)-RNP edited CD34+ hHSPCs</td><td>1 x 10⁶</td><td>15</td></tr><tr><td>4</td><td>Vehicle control</td><td>0</td><td>3</td></tr></table> N= 5/sex/group/donor/sacrificed time point for each donor for Groups 1-3 <ul style="list-style-type: none">Injection volume: 0.25 ml/mouse				Group	Study Agent	Dose Level (cells/mouse)	N/Sex/Group/Sacrificed time point	1	Unedited CD34+ hHSPCs	1 x 10 ⁶	15	2	CASGEVY	1 x 10 ⁶	15	3	(b) (4)-RNP edited CD34+ hHSPCs	1 x 10 ⁶	15	4	Vehicle control	0	3
Group	Study Agent	Dose Level (cells/mouse)	N/Sex/Group/Sacrificed time point																					
1	Unedited CD34+ hHSPCs	1 x 10 ⁶	15																					
2	CASGEVY	1 x 10 ⁶	15																					
3	(b) (4)-RNP edited CD34+ hHSPCs	1 x 10 ⁶	15																					
4	Vehicle control	0	3																					
Dosing Regimen	Single administration																							
Randomization	Yes (based on body weights)																							
Description of Masking	Not described																							
Scheduled Sacrifice Time Points	Weeks 8 and 20																							

Key Evaluations and Assessments:

- Mortality assessed twice daily
- Clinical observations taken twice daily
- Body weights (BW) measured on Days -7, -1, then every 6 days throughout the observation period; and prior to necropsy

Terminal procedures

- Percent chimerism at 8- and 20-weeks post-transplantation of human hCD45RA+ or hCD45+ cells¹² measured by flow cytometry in blood, spleen, and bone marrow for Groups 1, 2, and 3.
- BD¹³ and percent chimerism at 8- and 20-weeks post-transplantation of human DNA¹⁴ by (b) (4) for Groups 1 and 2.
- Chimerism at 8- and 20-weeks post-transplantation of hCD45+ cells by immunohistochemistry (IHC)¹⁵ for all Groups.
- On-target editing rate at 8- and 20-weeks post-transplantation in blood, spleen, and bone marrow by PCR/amplicon NGS¹⁶ for Groups 1 and 2.

Key Results:

- There were no consistent patterns of test article-related findings for mortality, clinical observations, or BW.
- Percentages of chimerism of transplanted cells in target tissues: There were no consistent patterns of genome editing-related findings. Groups 1-3 showed similar percentages of chimerism of transplanted cells in the whole blood, spleen, and bone marrow. The percentages of chimerism were lower at 20 weeks post-transplantation compared to 8 weeks.
- BD and percent chimerism of human DNA: There were no consistent patterns of genome editing-related findings. Groups 1 and 2 showed similar results with the highest percent chimerism of human DNA in bone marrow (12.8 to 25.4%), followed by spleen (6.2 to 7.7%), blood (0.79 to 3.6%), lung (1.8 to 2.3%), liver (1.2 to 1.8%), and kidney (0.6 to 1.6%); with low levels at the injection site, heart, mammary gland, jejunum, pancreas, brain, skeletal muscle; and minimal to below the level of quantification in prostate, uterus, ovary, and testis at Week 8. The percentage of chimerism in hematopoietic tissues (bone marrow, blood, and spleen) was lower at 20 weeks post-transplantation compared to 8 weeks.
- Chimerism of transplanted hCD45+ cells by IHC in non-target tissues: Groups 1-3 showed similar percentages of chimerism of human cells in all examined (non-target) tissues including testis. The percentage of chimerism was lower at 20 weeks post-transplantation compared to 8 weeks.
- On-target editing rate in blood, spleen, and bone marrow: Group 2 showed stable on-target indels throughout the 20-week study duration with similar on-target editing

¹² % Chimerism = number human cells/ (human cells + mouse cells) *100.

¹³ The list of tissues for BD include non-target tissues (brain, heart, injection site (tail), intestines, kidneys, liver, lung with bronchi, mammary glands (inguinal), ovaries, prostate, skeletal muscle (thigh), testes, pancreas, and uterus) and target hematopoietic tissues (bone marrow and spleen).

¹⁴ % Chimerism = human DNA detected per microgram total analyzed DNA

¹⁵ IHC was determined in non-target tissues only (brain, heart, injection site (tail), intestines, kidneys, liver, lung with bronchi, mammary glands (inguinal), ovaries, prostate, skeletal muscle (thigh), testes, pancreas, and uterus)

¹⁶ The on-target editing rates were based on %indels (indel reads over total reads).

frequency in bone marrow and spleen ($87.4 \pm 1.5\%$) at 8- and 20-weeks post-transplantation. Group 1 showed little to no indels in the spleen and bone marrow at 8- and 12-weeks post-transplantation.

Reviewer's Conclusion:

- Single IV administration of CTX001 from healthy donors in total body irradiated NSG mice, resulted in i) systemic chimerism of human cells determined by human DNA levels and flow cytometry and IHC for hCD45+ cells; ii) the highest level of chimerism in the bone marrow; and iii) stable on-target editing frequencies in spleen and bone marrow at 8- and 20-weeks post-transplantation of $87.4 \pm 1.5\%$, which is similar to the pre-transplantation editing frequency of 83.02%.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of CASGEVY following transplantation in various animal species.

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
23	A Pivotal Toxicity and Tumorigenicity Study Following a Single Intravenous Injection of Genetically Edited Compared to Unedited CD34+ Cells In Irradiated NOD/SCID/IL2R γ null (NSG) Mice	1016-2465

Note: Due to the exploratory nature of Study Report No. 2016-2315 entitled: 'An exploratory 55-day study for confirmation of tumor development following intravenous injection of HL-60 (human leukemia) cells in irradiated NOD/SCID/IL2R γ null (NSG) mice' and since CASGEVY was not administered, this study is not reviewed in this memo.

Study #23

Report Number	1016-2465
Date Report Signed	02/09/2018
Title	A Pivotal Toxicity and Tumorigenicity Study Following a Single Intravenous Injection of Genetically Edited Compared to Unedited CD34+ Cells in Irradiated NOD/SCID/IL2R γ null (NSG) Mice
GLP Status	<p>Yes</p> <p>The study was conducted in compliance with OECD Principles of GLP, except for the following:</p> <ul style="list-style-type: none"> • The (b) (4) analysis • Qualitative assessment of potentially malignant cells • Photographic equipment • Immunohistochemistry (IHC) and in-situ hybridization (ISH) evaluations

Testing Facility	(b) (4)																										
Objective(s)	To assess the toxicity and tumorigenicity of locus specific CRISPR/Cas9-gRNA genetically edited versus unedited CD34+ hHSPCs																										
Study Animals	Strain/Breed	NOD/SCID/IL2R γ null (NSG)																									
	Species	Mice																									
	Age	9 weeks old																									
	Body Weight	Males (23-32 g); females (17-24 g)																									
	#/sex/group	15/sex/group/sacrifice time point for Groups 1-3, 10/sex/group/sacrifice time point for Group 4, 5/sex/group/sacrifice time point for Group 5																									
	Total #	120																									
Conditioning	Total body irradiation 200 cGy at 160 cGy/min at Day 0																										
CD34+ hHSPCs from healthy donors	Donor #1 ((b) (6)), Donor #2 ((b) (6)), and Donor #3 ((b) (6)) (provided by (b) (4)) Note: Each study group contains three cohorts and each cohort evaluated control and test articles that were generated from each donor.																										
Test Article(s)	<ul style="list-style-type: none">CASGEVY (SPY101-RNP edited CD34+ hHSPCs) (cell viability - 80.5% to 90%)																										
Control Article(s)	<ul style="list-style-type: none">(b) (4)-RNP edited CD34+ hHSPCs (cell viability 86% to 88.5%)Unedited CD34+ hHSPCs (cell viability 93.5 to 95.5%)Vehicle injection buffer (PBS + 0.1% human serum albumin) (b) (4) gRNA is the same as in Report No. 2016-1955, that (b) (4).																										
Route of Administration	IV injection (0.25 mL/mouse) at Day 0																										
Description of the Disease/Injury Model and Implant Procedure	Healthy																										
Study Groups and Dose Levels	<table><tr><td>Group</td><td>Study Agent</td><td>Dose Level (cells/mouse)</td><td>N/Sex/Group/Sacrificed Time Point</td></tr><tr><td>1</td><td>Unedited CD34+ hHSPCs</td><td>1 x 10⁶</td><td>15</td></tr><tr><td>2</td><td>CASGEVY</td><td>1 x 10⁶</td><td>15</td></tr><tr><td>3</td><td>(b) (4)-RNP edited CD34+ hHSPCs</td><td>1 x 10⁶</td><td>15</td></tr><tr><td>4</td><td>Positive control: HL-60 human myelocytic leukemia cells</td><td>0.2 x 10⁶</td><td>10</td></tr><tr><td>5</td><td>Vehicle control</td><td>0</td><td>5</td></tr></table>			Group	Study Agent	Dose Level (cells/mouse)	N/Sex/Group/Sacrificed Time Point	1	Unedited CD34+ hHSPCs	1 x 10 ⁶	15	2	CASGEVY	1 x 10 ⁶	15	3	(b) (4)-RNP edited CD34+ hHSPCs	1 x 10 ⁶	15	4	Positive control: HL-60 human myelocytic leukemia cells	0.2 x 10 ⁶	10	5	Vehicle control	0	5
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	1	Unedited CD34+ hHSPCs	1 x 10 ⁶	15																							
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	5	Vehicle control	0	5																							
N= 5/sex/group/donor/sacrificed time point for each donor for Groups 1-3																											
Injection volume: 0.25 ml/mouse																											
Dosing Regimen	Single injection																										
Randomization	Yes																										
Description of Masking	Not described																										
Scheduled Sacrifice Time Points	Groups 1, 2, 3, and 5 at Week 20 (\pm 1 day) and Group 4 at Week 6																										

Key Evaluations and Assessments:

- Mortality/morbidity assessed twice daily
- Clinical observations taken twice daily

- BWs taken prior to Days -7, and -1 and post-dose every 6 days throughout the observation period
- Clinical pathology (hematology¹⁷, blood smears, and serum chemistry¹⁸) – prior to sacrifice
- Chimerism of human cells assessed by flow cytometry the in blood (hCD45RA+, mCD45+) – prior to sacrifice

Terminal procedures

- Organ weights¹⁹
- Gross and histopathology²⁰ examinations
- IHC for human CD44
- *In situ* hybridization (ISH) for mouse ICAM1 and human Alu for any identified tumor

Key Results:

- Mortality/morbidity: There were no test article related deaths.
- There were no consistent patterns of test article-related findings for clinical observations, BWs, hematology, blood smears, serum chemistry, organ weights, and gross pathology.
- Human cell chimerism in target hematopoietic tissues: Groups 1-3 showed human cell chimerism in blood (9-24% at 18 weeks and 6-9% at 20-week post-transplantation), in spleen (31-39% chimerism at 20 weeks post-transplantation) and in bone marrow (36-59% chimerism at 20 weeks post-transplantation).
- Histopathology: There were no consistent patterns of test article-related changes.
 - Groups 1-3 showed minimal to moderate infiltration/hyperplasia in spleen and thymus and minimal to moderate hyper-cellularity in bone marrow, while Groups 4 and 5 did not. Groups 1-3 and 5 showed no neoplasms while Group 4 (4/20) showed masses in subcutaneous tissue, abdominal area, axillary area or aorta. Groups 1-4 showed minimal-to-moderate increased myeloid-lineage cells in bone marrow and the origin of these cells could not be conclusively determined.

¹⁷ Hematology parameters include hematocrit, hemoglobin, hemoglobin distribution width, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, plateletcrit / thrombocrit, red blood cell count, red cell distribution width, reticulocyte counts (absolute and relative), white blood cell count (WBC), and WBC differential (absolute and relative).

¹⁸ Serum chemistry parameters include: A/G ratio (calculated), alanine aminotransferase, albumin (A), alkaline phosphatase, aspartate aminotransferase, bilirubin (total), total calcium, chloride, cholesterol (total), creatinine, globin (G) (calculated), glucose, phosphorus (inorganic), potassium, sodium, total protein, triglycerides, and urea.

¹⁹ A list of tissues for organ weight determination include: brain, lungs, spleen, heart, ovaries, testes, kidneys, prostate, thymus (when possible), liver, pancreas, and uterus.

²⁰ A list of tissues for microscopic examination include: gross lesions, adrenals, aorta (thoracic), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur with marrow, gallbladder, heart, injection site, ileum, jejunum, kidneys, liver, lungs with bronchi, lymph nodes (iliac and inguinal), optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin, subcutis & mammary gland (inguinal), spinal cord (cervical), spleen, sternum and marrow, stomach, testes, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus, and vagina.

Reviewer's Conclusion:

- A single IV injection of 1×10^6 CASGEVY/mouse in total body irradiated NSG mice did not result in any significant adverse findings or tumor formation throughout the 20-week study duration post-transplantation.

Genotoxicity Studies:

Study Number	Study Title / Publication Citation	Report Number
24	Application of GUIDE-seq to SPY101 Guide RNA in CD34+ Hematopoietic Stem and Progenitor Cells to Reveal Candidate Off-Target Sites	CTxSR-016
25	SPY101 Off-Target Assessment by Hybrid Capture Sequencing	CTxSR-015
26	Characterization of SPY101-edited Good Laboratory Practice (GLP) Samples -- Off- Target Editing Assessment by Hybrid Capture Sequencing	CTxSR-024
27	Application of GUIDE-seq to SPY101-RNP in CD34+ Hematopoietic Stem and Progenitor Cells in Order to Identify Candidate Off-Target Sites	RES-IND-041
28	SPY101 Off-Target Assessment by Hybrid Capture Sequencing	RES-IND-042
29	Application of GUIDE-seq to SPY101-RNP in CD34+ Hematopoietic Stem and Progenitor Cells to Identify Candidate Off-Target Regions in Patient Samples	R263
30	Off-Target Editing Assessment in Patient Samples by Hybrid Capture Sequencing	R264
31	Evaluation of CRISPR-Cas9-Mediated Gene Editing on Chromosomal Aberration Using Karyotyping Analysis	CTxSR-019
32	Further Characterization of Editing Outcomes at the SPY101 Cas9 Editing Site	CTxSR-018
33	Reproducibility of Assays Used to Measure Editing Outcomes at the SPY101 Cas9 Editing Site	CTxSR-040
34	Characterization of CTX001 GMP Process Quality (PQ) Lots (b) (4) -- Off-Target and On-Target Editing Assessment by Hybrid Capture Sequencing	CTxSR-028
35	Characterization of CTX001 GMP Process Qualification (PQ) Lots (b) (4) -- Off-Target and On-Target Editing Assessment by Hybrid Capture Sequencing	CTxSR-036

Off-target genome editing

Review of the above listed off-target genome editing studies (Numbers 24-30), was performed by the Bioinformatics reviewer. Please see the Bioinformatics review memo for details. Briefly, the candidate genome editing off-target sites of CTX001 were nominated by in silico analysis and non-biased GUIDE-seq analysis. The confirmation of the nominated candidate off-target sites was performed on CTX001 manufactured from healthy donors, and patients with SCD or TDT by hybrid capture Next-Generation Sequencing (NSG) analysis. CTX001 showed no editing at the off-target sites evaluated.

Chromosomal aberration

- **Study #31** (Report No. CTxSR-019): Karyotyping analysis of CTX001 manufactured from three healthy donors showed no SPY101-RNP editing-related abnormal findings. Due to the limitations of karyotyping analysis for detection of chromosomal aberrations that are smaller than 5 Mb in size, additional analyses of chromosomal aberration were

performed by long-range PCR sequencing analysis and hybrid capture sequencing analysis in Report No. CTxSR-018 below.

- **Study #32** (Report No. CTxSR-018): Long-range PCR-sequencing analysis of CTX001 manufactured from three healthy donors showed i) an average 88% on-target editing frequency; ii) an average 5.1% on-target large insertion frequency, defined as insertion >30bp DNA sequence iii) an average 5.1% on-target large deletion frequency, defined as deletion >30bp DNA sequence; iv) the maximum size of an on-target large insertion was 4986(±595) bp, v) the median size of an on-target large insertion was 508(±27) bp; v) the maximum size of an on-target large deletion was 7362(±62) bp, and vi) the median size of an on-target large deletion was 801(±71) bp. Hybrid capture sequencing analysis of CTX001 from the same donors showed similar frequencies of on-target editing, on-target large insertions, and on-target large deletions compared to long-range PCR-sequencing analysis. Based on considerations that i) hybrid capture sequencing can detect inter-chromosomal translocation while long-range capture sequencing cannot, and ii) similar frequencies of on-target large insertions were shown by hybrid capture sequencing and long-range PCR sequencing analyses, results showed no consistent patterns of CTX001 related inter-chromosomal translocation.
- **Study #33** (Report No. CTxSR-040): Long-range PCR sequencing and hybrid capture sequencing analyses of CTX001 manufactured from one healthy donor showed similar frequencies for on-target editing, on-target large deletions, and on-target large insertions.
- **Study #34** (Report No. CTxSR-028): Hybrid capture sequencing analysis of CTX001 from three cGMP PQ lots from three healthy donors ((b) (6)) manufactured by ((b) (4)) based on 857 candidate off-target sites nominated by GUIDE-seq, described in Report No. CTxSR-016, and on-target methods, as described in Report No. CTx-018, resulted in: i) no detectable SPY101-RNP related off-target editing, and ii) low frequency of on-target large insertions [8(±1.3)%], iii) low frequency of on-target large deletions [5.1(±1.1)%], iv) higher frequency of on-target small insertions [29.3(±3.8)%], defined as an insertion ≤30bp DNA sequence, and v) higher frequency of on-target small deletions [46.3(±1.8)%], defined as deletion ≤30bp DNA sequence.
- **Study #35** (Report No. CTxSR-036): Hybrid capture sequencing analysis of CTX001 from four cGMP PQ lots that were manufactured from three healthy donors ((b) (6)) by ((b) (4)) based on a combined 7101 candidate off-target sites (5007 sites by in silico homology search and 2094 sites by GUIDE-seq) showed i) no detectable SPY101-RNP-related off-target editing at 1 % difference threshold, ii) low frequency of on-target large insertion [5(±2.2)%], iii) low frequency of on-target large deletion [5.5(±1.3)%], iv) high frequency of on-target small insertions [32.7(±3.2)%], and v) high frequency of on-target small deletions [47.9(±5.0)%].

Reviewer's Conclusion:

- CTX001 manufactured from healthy donors showed no detectable chromosomal aberration by karyotyping, long-range PCR-sequencing analysis, and hybrid capture NSG analysis.
- Assessment of chromosomal aberrations for CTX001 manufactured from patients with SCD or TDT was not performed. It is unclear whether genetic heterogeneity or patient-specific factors could contribute to CTX001-related chromosomal aberrations, which may warrant additional monitoring.

Developmental and Reproductive Toxicology (DART) Studies:

No DART studies were conducted with CTX001. The applicant's rationale for not conducting DART studies include: i) no CTX001-related pathologic findings were observed in reproductive organs in the completed toxicology study (Study Report No. 1016-2465), ii) very low distribution of CTX001 to reproductive organs was reported in the completed biodistribution study (Study Report No. 1016-2475), iii) CTX001 is an ex vivo genome edited autologous CD34+ hHSPCs via a specific CRISPR Cas9/SPY101 gene editing approach, and iv) the manufacturing of CTX001 does not involve an integrating vector.

Reviewer comment: This is acceptable based on the product characteristics and safety profile.

Carcinogenicity/Tumorigenicity Studies:

No carcinogenicity studies were conducted with CTX001.

Other Safety/Toxicology Studies

The following immunogenicity study was conducted with CTX001.

Study Number	Study Title / Publication Citation	Report Number
36	Evaluation of Innate Immune Response Upon Treatment of Primary Human CD34+ Cells With SPY101 gRNA and Cas9 Protein	CTxSR-013

Overview of Immunogenicity Study

Study #36

Study Report Number: CTxSR-013

Study Title: Evaluation of Innate Immune Response Upon Treatment of Primary Human CD34+ Cells with SPY101 gRNA and Cas9 Protein

Objective: To determine whether CTX001 manufactured from mPB from a healthy donor could lead to innate immune activation and/or negatively influence cell number and/or growth.

Study Agents:

- Test article: SPY101-RNP
- Negative control article: EGFP-RNP

- Negative control article: Cas9 protein alone
- Positive control: R848 (an imidazoquinoline that stimulated nuclear factor (NF)-Kb activity and cytokine transcription which has been observed in peripheral blood mononuclear cells (PBMCs) ²¹

Methods: CD34+ hHSPCs (2.5×10^6 cells/group) from a healthy donor (b) (6) were incubated with the Cas9-gRNA RNP complex ((b) (4) Cas9 [(b) (4)] and (b) (4) gRNA), followed by EP same as described in Report No. CTxSR-010. Cells were harvested at (b) (4) post-EP, and cell viability was determined. PBMCs (2.5×10^6 cells/group) and CD34+ hHSPCs (2.5×10^6 cells/group) treated with R848 (10 µg/mL) served as positive control groups.

- Expression of innate immune stimulation-related genes including induced proteins with tetratricopeptides repeats 1 (IFIT1), retinoic acid-inducible gene 1 (RIG1), 2'-5'-oligoadenylate synthetase 1 (OAS1), and interleukin 6 (IL-6) transcripts, determined by (b) (4).

Key Results:

- The positive control R848 PBMC group showed high levels of expression of IFIT1, TIG1, OAS1, and IL-6 transcripts during (b) (4) post-EP. However, the positive control of R848 CD34+ cells did not.
- BCL11A/SPY101, EGFP, Cas9 only, and unedited groups showed low levels of expression of IFIT1, TIG1, OAS1, and IL-6 transcripts during (b) (4) post-EP.

Reviewer's Conclusion:

- The study results were uninterpretable since the positive control group of R848 CD34+ cells did not show high levels of expression of IFIT1, TIG1, OAS1, and IL-6 transcripts, which were observed in R848 PBMC cohort.

APPLICANT'S PROPOSED LABEL

- Section 13 ('Nonclinical Toxicology') should be revised to include only the necessary nonclinical information needed for safe use of the product.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of this biologics license application

²¹ Marion J, et al. (2002) Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. Nat Immunol 3(6): 499-499.

KEY WORDS/TERMS

CASGEVY, exagamglogene autotemcel, exa-cel, CTX001, autologous CD34+ hHSPCs, CRISPR/Cas9 genome editing, spCas9 protein, SPY101 gRNA, IV administration, sickle cell disease, beta-thalassemia