

I concur with this review memo. 8/22/22 I Wu

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER: STN #125755.000

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PRODUCT: SKYSONA (elivaldogene autotemcel)

APPLICANT: bluebird bio, Inc.

PROPOSED INDICATION: For the treatment of patients less than 18 years of age with early cerebral adrenoleukodystrophy (CALD) who do not have an available and willing human leukocyte antigen (HLA)-matched sibling hematopoietic stem cell (HSC) donor.

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EXECUTIVE SUMMARY:

Elivaldogene autotemcel (eli-cel) is comprised of autologous cluster of differentiation (CD)34-positive (CD34+) hematopoietic stem cells (HSCs) that have been transduced with Lenti-D, a lentiviral vector (LVV) encoding *ABCD1* complementary DNA (cDNA), that is intended for intravenous (IV) administration in pediatric patients with early cerebral adrenoleukodystrophy (CALD).

In vitro pharmacology studies were conducted with healthy human donor CD34+ HSCs, CD34+ HSCs obtained from patients with adrenomyeloneuropathy (AMN), and adrenoleukodystrophy protein (ALDP)-deficient fibroblasts obtained from patients with CALD, transduced using the Lenti-D vector used in eli-cel. These studies demonstrated that vector-driven *ABCD1* transgene expression and ALDP production resulted in improvements in very-long-chain fatty acid (VLCFA) metabolism in CALD fibroblasts and AMN patient CD34+ HSCs. In vivo assessment of Lenti-D-transduced healthy donor derived CD34+ HSCs transplanted in myeloablated immunodeficient mice demonstrated bone marrow and brain engraftment that was associated with stable *ABCD1* transgene expression and ALDP production for the 3-month study duration.

A 92-day GLP toxicology study in myeloablated (b) (4) mice evaluated a single administration of 1×10^6 Lenti-D transduced healthy human CD34+ HSCs/mouse. There were early mortalities in both the test article (Lenti-D transduced CD34+ HSCs) and control (non-transduced CD34+ HSCs) groups. Although the cause of death was undetermined, the early mortalities occurred at a higher frequency in the control group and human cell engraftment was confirmed in both groups. No other test article related toxicities were observed in this study. Although a true no observed adverse effect level (NOAEL) could not be determined, the dose level administered in this study, approximately 5×10^7 CD34+ cells/kg, represents the maximum feasible dose and a 10-fold multiple of the minimum recommended dose level for patients with CALD ($\geq 5 \times 10^6$ CD34+ cells/kg).

Traditional carcinogenicity studies were not conducted for eli-cel. However, integration site analysis (ISA) was performed on Lenti-D transduced pre-transplant human CD34+ HSCs and post-transplant engrafted bone marrow cells (BMCs) from animals in the GLP toxicology study harvested at 29 and 92 days. Lenti-D transduced HSCs demonstrated the expected integration profile for self-inactivating lentiviral vectors, with preferred integration in gene-coding regions across the whole genome, no preference for integration in transcriptional start sites, and no bias for the 5' or 3' end of genes. There were no notable differences between the pre- and post-transplant samples.

An in vitro immortalization (IVIM) assay was conducted with mouse lineage-depleted (hematopoietic non-lineage committed; Lin-) BMCs (the murine equivalent of human CD34+ HSCs) transduced with Lenti-D. There was a reduced potential for immortalization induced by insertional mutagenesis of the clinical vector, Lenti-D, compared to positive control vectors.

Reproductive and developmental toxicity studies were not performed for eli-cel which is acceptable based on the product characteristics and safety profile.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

Based on review of the nonclinical data in this BLA submission (STN #125755), there are no nonclinical deficiencies identified in the pharmacology/toxicology studies. There are no requests for further nonclinical testing of elivaldogene autotemcel at this time. The nonclinical data provided in this BLA submission support the approval of the licensure application.

Formulation and Chemistry:

Elivaldogene autotemcel (eli-cel) is a cell suspension for intravenous (IV) infusion. Its active substance consists of autologous CD34+ cells from patients with cerebral adrenoleukodystrophy (CALD) transduced with the Lenti-D lentiviral vector (LVV) that encodes the human *ABCD1* transgene (cDNA) and expresses human adrenoleukodystrophy protein (ALDP). A single dose of eli-cel contains a minimum of 5.0×10^6 CD34+ cells/kg of body weight, suspended in a solution containing 5% dimethyl sulfoxide (DMSO).

Related File

IND #15433: bluebird bio, Inc.; Autologous Hematopoietic Stem Cells (CD34+, (b) (4)) Transduced with Lentiviral Vector, Lenti-D, Encoding the Human ATP-Binding Cassette, Sub-family D, Member 1 (ABCD1) Gene; Cultured with (b) (4) Following Mobilization with Filgrastim; IV; Treatment of childhood cerebral adrenoleukodystrophy (CCALD) in boys 15 years old and younger

Abbreviations

<i>ABCD1</i>	Adenosine triphosphate [ATP]-binding cassette, sub-family D, member 1
ALD	Adrenoleukodystrophy
ALDP	adrenoleukodystrophy protein
AMN	Adrenomyeloneuropathy
ATP	Adenosine triphosphate
BMC	Bone marrow cells
CALD	Cerebral adrenoleukodystrophy
CD	Cluster of differentiation
CFU	Colony forming unit
cDNA	Complementary deoxyribonucleic acid
gDNA	Genomic deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
G-CSF	Granulocyte-colony stimulating factor
GLP	Good laboratory practice
GVHD	Graft versus host disease
HEK293T	Human embryonic kidney 293T cells
HIV	Human immunodeficiency virus
HPC-A	Hematopoietic progenitor cells obtained by apheresis
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
IS	Integration site
ISA	Integration site analysis
IV	Intravenous
IVIM	In vitro immortalization
LAM-PCR	Linear - amplification mediated PCR
(b) (4)	
LVV	Lentiviral vector
MDS	Myelodysplastic syndrome
(b) (4)	
MND	Motor neuron disease
MNDU3	Murine leukemia virus-derived MND promoter
MOI	Multiplicity of infection
NOAEL	No observed adverse effect level
(b) (4)	
PCR	Polymerase chain reaction
qPCR	Quantitative PCR
SIN	Self-inactivating
VCN	Vector copy number
VLCFA	Very long-chain fatty acid

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INTRODUCTION

Adrenoleukodystrophy (ALD) is a rare, X-linked disease caused by mutations in the ATP-binding cassette, sub-family D, member 1 (*ABCD1*) gene which encodes adrenoleukodystrophy protein (ALDP)(1). ALDP is a peroxisomal transport protein involved in the degradation of very-long chain fatty acids (VLCFAs)(2). Absent or dysfunctional ALDP results in accumulation of VLCFAs, primarily in the adrenal glands and white matter of the brain and spinal cord. Cerebral ALD (CALD), the most severe manifestation of ALD, affects approximately 40% of boys with ALD, typically during childhood. CALD is characterized by rapidly progressive cerebral demyelination leading to progressive, irreversible loss of neurologic function and death (2).

Currently, there is no FDA-approved treatment for CALD. The standard of care of children with CALD is allogeneic hematopoietic stem cell transplantation (allo-HSCT). Allo-HSCT is thought to enable migration of donor-derived cells into the brain, which results in donor-derived macrophages and/or microglial cells that express ALDP, thereby allowing the local degradation of VLCFAs and a decrease or stopping of demyelination (3). Although allo-HSCT can arrest disease progression if performed at the early stage of cerebral involvement, it has significant limitations including donor availability and transplant-related risks such as graft-versus-host disease (GVHD). Ideally, allo-HSCT is performed using an HLA-matched sibling hematopoietic stem cell (HSC) donor. However, matched sibling donors are available for <30% of patients (4).

Alternatives include transplantation with cells derived from an HLA-mismatched related donor, or from an HLA-matched or -mismatched unrelated donor which has a higher risk of complications.

Eli-cel is an autologous HSC product that is transduced with a lentiviral vector (LVV) encoding *ABCD1* cDNA for production of functional human ALDP. The proposed mechanism of action is that after eli-cel is infused, transduced CD34⁺ HSCs engraft in the bone marrow and subsequently differentiate into various cell types, including monocytes that migrate to the brain where they further differentiate into long-lived macrophages and cerebral microglia that can produce functional ALDP and replace deficient microglial cells (5-7). The functional ALDP can then enable the local degradation of VLCFAs in the brain, which in turn can stabilize the disease by preventing further inflammation and demyelination.

NONCLINICAL STUDIES

The applicant was not able to obtain autologous CD34⁺ cells from CALD patients, and thus the final clinical product was not used in the nonclinical studies summarized in this memo. However, they were able to obtain CD34⁺ HSCs from a limited number of subjects with adrenomyeloneuropathy (AMN), a less severe form of ALD also resulting from a loss of function *ABCD1* gene mutation. These ALDP-deficient AMN CD34⁺ HSCs were used as a surrogate product in the in vitro pharmacology studies to demonstrate vector integration, ALDP production, and correction of VLCFA metabolism. Additionally, ALDP-deficient fibroblasts from subjects with CALD and CD34⁺ HSCs from healthy donors were used in these in vitro studies.

The applicant also stated that it was not possible to obtain sufficient quantities of AMN CD34⁺ HSCs to be used in in vivo studies. Therefore, CD34⁺ HSCs from healthy donors (with normal ALDP expression) were used for the in vivo pharmacology/toxicology studies.

All cells were transduced with the same LVV, Lenti-D, that is used to produce eli-cel (**Figure 1**). The Lenti-D LVV is a replication-defective, self-inactivating (SIN), HIV-1-based LVV that is pseudotyped with VSV-G and utilizes an internal MNDU3 promoter to control expression of the human *ABCD1* transgene that encodes human ALDP.

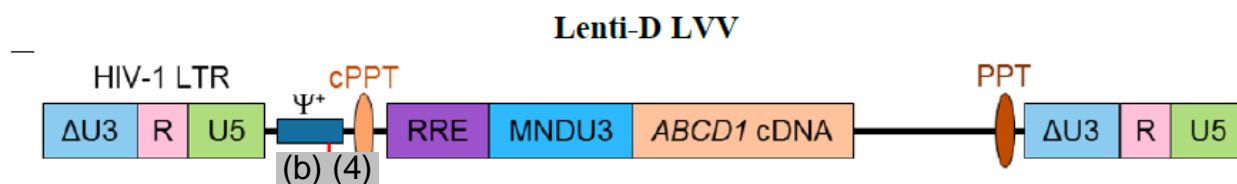


Figure 1. Lenti-D vector design. The integrated Lenti-D proviral (i.e., transgenic) cDNA is flanked by identical human immunodeficiency virus-1 (HIV-1)-based 5' and 3' long terminal repeats (LTRs) each containing wildtype repeat (R) and unique 5 (U5) regions, but with modified unique 3 (U3) regions (ΔU3). The ΔU3 regions contain a deletion of the U3 enhancer/promoter that confers the self-inactivating (SIN) property. The extended packaging signal (Ψ⁺), central polypurine tract (cPPT) and Rev-response element (RRE) are incorporated to facilitate retroviral packaging, reverse transcription, and nuclear export of the viral RNA genome, respectively.

(b) (4). Expression of the *ABCD1* transgene, encoding the adrenoleukodystrophy protein (ALDP), is under local control of the internal MNDU3 promoter which consists of only the U3 enhancer/promoter region from the murine myeloproliferative sarcoma virus (MPSV) LTR, modified by deletion of the negative control region (NCR).

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

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

In Vitro Studies

Study Number	Study Title	Report Number
1	(b) (4)	NC-12-001
2	(b) (4)	NC-12-003
3	Functional Correction of ALDP Deficiency in ALD-defective Primary Human Fibroblasts	NC-12-004
4	(b) (4)	NC-12-045
5	(b) (4)	NC-12-050
6	(b) (4)	NC-12-051
7	(b) (4)	NC-12-058
8	Large-scale Transduction of GCSF-mobilized Normal CD34+ Cells with Lenti-D for a Single-dose Toxicology and Biodistribution Study in (b) (4) Mice	NC-12-012

In Vivo Studies

In Vivo Studies in Healthy Animals

Study Number	Study Title	Report Number
9	Evaluation of Bone Marrow Engraftment of Lenti-D Lentiviral Vector Transduced CD34+ Cells and Subsequent Migration to Murine Brain Tissue	B1-13-003

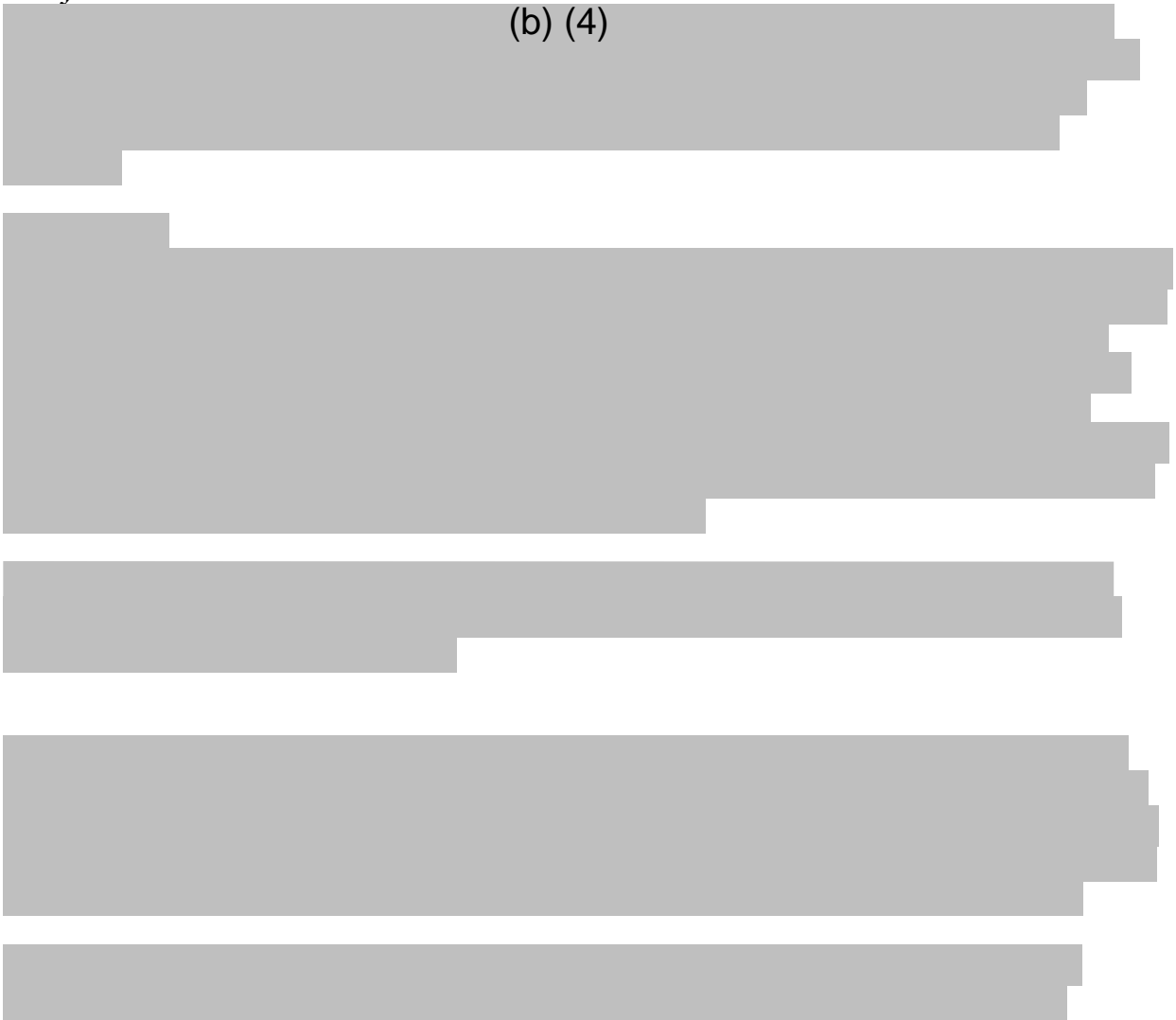
Note: Study Nos. 1-9 are briefly summarized in this review memo under ‘Overview of Pharmacology Studies.’

Reviewer Comment:

There are no known animal models of CALD that recapitulate the human disease. Murine ABCD1/ALDP knockout mice (ALD mice) lack ALDP and have altered VLCFA metabolism, but the disease phenotype resembles AMN rather than ALD and does not display the cerebral inflammation and cerebral demyelination observed in human cerebral ALD (CALD).(8) This reviewer agrees with the applicant that this model is not suitable to demonstrate resolution of cerebral inflammation and demyelination in an animal model of CALD by gene therapy-mediated ABCD1 transgene expression of ALDP.

Overview of Pharmacology Studies**Overview of In Vitro Studies****Study #1**

(b) (4)



(b) (4)

Study #2

(b) (4)

Study #3

Study objective: This study was performed to evaluate the relationship between Lenti-D MOI, VCN, and ALDP expression levels in ALDP-deficient human fibroblasts.

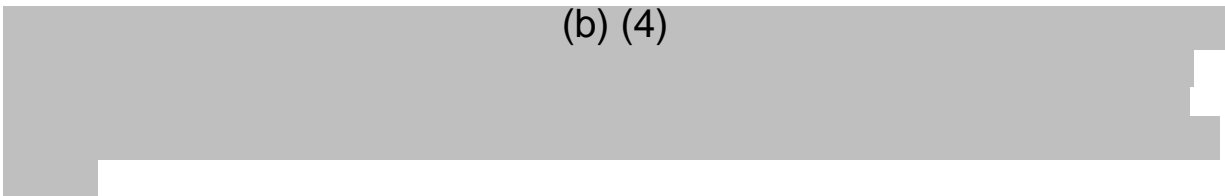
Study design: ALDP-deficient human fibroblasts were transduced with (b) (4) different lots of Lenti-D vector at MOIs ranging from (b) (4) or with a control vector containing only the MND promoter (i.e., no transgene). Untransduced cell lines with normal levels of ALDP expression were included as comparative controls (b) (4) was used to measure changes in levels of the VLCFA C26:0-lyso-PC in mock-transduced and Lenti-D vector-transduced cells. ALDP expression and average VCN in the transduced cells were determined by flow cytometry and qPCR, respectively. All analyses were performed on cell collected day (b) (4) post-transduction.

Results: Lenti-D vector transduced cells were shown to express ALDP. Transductions conducted at (b) (4). VLCFA levels in ALDP-deficient human fibroblasts were reduced following transduction with the Lenti-D vector at all MOIs evaluated. Transduced cells with an average VCN of (b) (4) showed complete correction of VLCFA accumulation, comparable to the level of cells with a normal ALDP expression phenotype (b) (4). There was no correction in VLCFA levels in the mock-transduced cells.

Study #4


(b) (4)

(b) (4)

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Study #5 and Study #6

(b) (4)

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Study #7

(b) (4)

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(b) (4)

Study #8

(b) (4)

(b) (4)

Overview of In Vivo Studies**In Vivo Studies in Healthy Animals****Study #9**

Report Number		B1-13-003
Date Report Signed		10-July-2015
Title		An Investigative Study to Evaluate Lenti-D ABCD1-transduced CD34+ Human Hematopoietic Stem Cell Engraftment in Bone Marrow and Subsequent Migration to Brain Tissue in Myeloablated (b) (4) Mice
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To evaluate whether Lenti-D lentiviral vector (LVV)-transduced human CD34+ hematopoietic stem cells (HSCs) administered to immunodeficient, myeloablated mice would result in bone marrow engraftment and the subsequent presence of human-origin Lenti-D LVV-transduced microglial cells in brain tissue.
Study Animals	Strain/Breed	(b) (4)
	Species	Mouse
	Age	6 weeks
	Body Weight	15 – 25 g
	#/sex/group	5-10 female mice/group
	Total #	30
Test Article(s)		G-CSF mobilized normal human CD34+ HSCs transduced with Lenti-D LVV
Control Article(s)		G-CSF mobilized normal human CD34+ HSCs mock-transduced
Route of Administration		Intravenous (IV)
Description of the Administration Procedure		All mice received busulfan (30 mg/kg) via intraperitoneal (IP) injection for myeloablation on Day 0 of Week 1, one day prior to administration of the HSCs. On Day 1 of Week 1, mice were injected IV via tail vein with either mock-transduced CD34+ HSCs or Lenti-D LVV-transduced CD34+ HSCs.

	GROUP	MOUSE STRAIN	TEST OR CONTROL ARTICLE	NO. ANIMALS	DOSE LEVEL (CELLS/MOUSE)
Study Groups and Dose Levels	1	(b) (4)	Mock-transduced CD34+ HSCs	5	1x10 ⁶
	2	(b) (4)	Lenti-D LVV-transduced CD34+ HSCs	10	1x10 ⁶
	3	(b) (4)	Mock-transduced CD34+ HSCs	5	1x10 ⁶
	4	(b) (4)	Lenti-D LVV-transduced CD34+ HSCs	10	1x10 ⁶
Source: Modified from study report b1-13-003, page 16 of 162					
Dosing Regimen	Single administration				
Randomization	Yes				
Description of Masking	Not provided in the study report				
Scheduled Sacrifice Time Points	Week 9 (n=1 animal from Groups 1 and 3 and n=2 animals from Groups 2 and 4) Week 13 (all remaining animals)				

Reviewer Comment: Both mouse strains used in this study are immunodeficient to allow for the engraftment of human CD34+ HSCs. (b) (4) mice promote predominantly lymphoid cell development. (b) (4) mice additionally express several myelosupportive transgenic human cytokines (interleukin-3 [IL-3], granulocyte/macrophage-colony stimulating factor [GM-CSF] and steel factor [SF]) which aid in human myeloid cell expansion. The applicant hypothesized that, relative to the (b) (4) mice, the (b) (4) mice might allow enhanced differentiation of myeloid cells from the engrafted human HSCs, and perhaps enhanced differentiation to brain microglial cells.

Key Evaluations and Assessments:

- Cage-side/clinical observations were performed daily for each animal.
- Body weights were recorded weekly.
- Non-terminal blood samples were collected by retro-orbital bleed at Week 8 from all animals to determine human cell engraftment and VCN.
- Euthanasia was performed on day of scheduled sacrifice. Terminal blood was collected by cardiac puncture from animals in the Week 13 sacrifice group only. Clinical pathology, including hematology and clinical chemistry, was performed.
- A complete necropsy was not performed, but selected tissues were collected including the brain and left and right femurs from each animal. Organ weights were recorded.

- Cell engraftment and VCN were evaluated in blood, bone marrow and brain tissue collected from both Week 9 and Week 13 sacrificed animals.

Key Results:

Clinical observations: There were no early mortalities on this study and no test-article related clinical observations.

Body weight: There were mild test article-related effects on body weights. From Week 10 to Week 13, (b) (4) mice administered mock-transduced or Lenti-D-transduced cells gained 2.2 and 1.0 g (9.4 and 4.4%, respectively). (b) (4) administered mock-transduced or Lenti-D-transduced cells mice lost 2.0 and 2.4 g (-8.0% or -10.0%, respectively). Thus, after Week 10, mice receiving Lenti-D transduced HSCs tended to gain slightly less weight (-52.9% in (b) (4) mice) or to lose slightly more weight (-25.0% in (b) (4) mice) than those receiving mock-transduced HSCs. At Week 13, (b) (4) mice receiving Lenti-D transduced HSCs had slightly (3.9% or 5.2%, respectively) lower body weights than mock-transduced controls.

Clinical pathology: There were moderate test article-related effects on platelet counts and differences between the strains. At Week 13, mean platelet counts for (b) (4) mice receiving mock- or Lenti-D transduced HSCs were 563 and 295 K/ μ L, respectively. For (b) (4) mice receiving mock- or Lenti-D transduced HSCs, mean platelet counts were 347 and 208 M/ μ L, respectively. Thus, platelet counts were lower for mice receiving Lenti-D transduced HSCs relative to mock-transduced HSCs regardless of strain.

Bone marrow engraftment: Engraftment of human CD34+ HSCs within bone marrow was demonstrated in all mice in all groups as measured by the percentage of human-origin hematopoiesis-derived cells (hCD45+ leukocytes). At Week 13, the mean percentage of hCD45+ cells in bone marrow was marginally but not significantly higher for mice receiving Lenti-D transduced HSCs compared to mock transduced. (b) (4) mice receiving Lenti-D transduced cells had 37.7% hCD45+ cells in the bone marrow versus 30.3% in mice receiving mock transduced cells. (b) (4) mice receiving Lenti-D transduced HSCs had 40.3% hCD45+ cells in the bone marrow versus 33.7% in mice receiving mock transduced HSCs.

There were strain-related differences in the percentage of human-origin cells of myeloid lineage (hCD45+/hCD33+ monocytes and neutrophils) within the bone marrow of mice. At Week 13, the mean value for %hCD45+/hCD33+ cells in the bone marrow of (b) (4) mice receiving Lenti-D transduced HSCs was 13.6%. In (b) (4) mice receiving Lenti-D transduced HSCs the mean value for %hCD45+/hCD33+ cells in the bone marrow was 84.7%. These data suggest that the engrafted HSCs differentiated predominantly toward CD33+ myeloid lineages within bone marrow in the (b) (4) mice.

Bone marrow ALDP expression: At Week 13, the mean %hCD45+/hALDP+ cells in bone marrow of mice receiving mock-transduced HSCs was 3.0% in (b) (4) mice and 3.1% in (b) (4) mice. In mice receiving Lenti-D transduced HSCs, the mean %hCD45+/hALDP+ cells in bone marrow was increased to 6.1% in (b) (4) mice and 19.8% in (b) (4) mice.

Bone marrow VCN: At Week 13, the mean VCN values in bone marrow of (b) (4) mice receiving mock-transduced HSCs were 0.0017 copies/diploid genome for (b) (4) mice and 0.0269 copies/diploid genome for (b) (4) mice. In mice receiving Lenti-D transduced HSCs the mean VCN values in bone marrow were 0.5438 copies/diploid genome for (b) (4) mice and 0.8543 copies/diploid genome for (b) (4) mice. The higher VCN values observed in (b) (4) mice receiving Lenti-D transduced HSCs is consistent with the greater ALDP expression in this group.

Peripheral blood engraftment: At Week 13, the mean %hCD45+ cells in peripheral blood of (b) (4) mice was 11.5% in mice receiving mock transduced HSCs and 12.7% in mice receiving Lenti-D transduced HSCs. For (b) (4) mice, the mean %hCD45+ cells in peripheral blood was 6.5% for mock transduced HSCs and 17.2% for Lenti-D transduced HSCs.

Similar to results seen in the bone marrow, at Week 13 there were strain-related differences in the percentage of human-origin cells of myeloid lineage within peripheral blood of mice. In (b) (4) mice receiving Lenti-D transduced cells the mean value for %hCD45+/hCD33+ cells was 8.6% compared to 48.0% in (b) (4) mice. The relative numbers of %hCD45+/hCD33+ cells were lower in peripheral blood than in bone marrow. While there were no differences in the hCD45+/hCD19+ B cells in peripheral blood of (b) (4) mice, (b) (4) mice had moderately higher %hCD45+/hCD3+ T cells than (b) (4) mice (15.8% versus 7.1%, respectively in Lenti-D transduced HSC administered animals). Thus, the engrafted HSCs differentiated predominantly toward CD33+ myeloid lineages and CD3+ T cells within peripheral blood in the (b) (4) mice.

Peripheral blood VCN: At Week 13, the mean VCN values in PBMCs of mice receiving mock-transduced HSCs were 0.2243 copies /diploid genome for (b) (4) mice and 0.2767 copies/diploid genome for (b) (4) mice. The mean VCN for mice receiving Lenti-D transduced HSCs were 0.6360 copies/diploid genome for (b) (4) mice and 0.9771 copies/diploid genome for (b) (4) mice.

Brain engraftment: Engraftment of human HSC-derived cells in brain tissue was observed only within (b) (4) mice receiving mock transduced or Lenti-D transduced HSCs. hCD45+/hIba-1+/hFGFR-1+ round mononuclear cells were observed within the meninges, choroid plexus and brain parenchyma. The relative number of engrafted cells was similar in (b) (4) mice receiving mock transduced or Lenti-D transduced HSCs. However, it was not possible to demonstrate specific staining of hALDP+ cells within (b) (4) mouse brain tissue. Despite the absence of hALDP+ staining, (b) (4) mice receiving either mock transduced or Lenti-D transduced HSCs demonstrated brain engraftment of mononuclear cells that were human-origin hematopoiesis-derived cells (i.e., hCD45+ leukocytes), with a human-origin microglial cell immunophenotype (hIba-1+), and expressing a unique human microglial cytoplasmic protein (FGFR-1+) that was not present in normal mouse brain.

Reviewer Comment:

- *Per the study report, the antibodies used to detect hALDP expression in the control human brain tissue were the same as those used to detect hALDP expression in the mouse samples from this study and the same as those validated in published literature (9, 10). The reason for lack of hALDP staining in (b) (4) mice, despite the presence of HSC-derived cells within the mouse brain tissue, is unknown.*

Organ weights: At Week 13, mean brain weights for (b) (4) mice receiving mock transduced or Lenti-D transduced HSCs were 0.5133 or 0.5074 g, respectively, and for (b) (4) mice receiving mock transduced or Lenti-D transduced HSCs were 0.5046 or 0.4567 g, respectively. Thus, (b) (4) mice receiving Lenti-D transduced HSCs had slightly (10.0%) lower brain weights. This is consistent with the lowest mean body weights (greatest body weight loss) in this group.

Conclusions: This investigational study demonstrated that Lenti-D transduced healthy human donor CD34+ HSCs administered to immunodeficient, myeloablated (b) (4) mice resulted in engraftment of human-origin cells within bone marrow and peripheral blood of both strains of mice, with subsequent brain engraftment of human CD45+ cells with a microglial (hIba-1+) immunophenotype and expressing a unique human microglial cell cytoplasmic protein (FGFR-1+) within (b) (4) mouse brain tissue.

SAFETY PHARMACOLOGY STUDIES

Safety pharmacology studies were not conducted for this product.

PHARMACOKINETIC STUDIES (Cell Distribution)

Assessment of the cell distribution profile of eli-cel was incorporated in the toxicology study. Therefore, these data are summarized with the respective toxicology study (Study #10).

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of eli-cel following administration in mice.

Toxicology Study:

Study Number	Study Title / Publication Citation	Report Number
10	Single Dose Toxicity and Biodistribution Study of Lenti-D in Mice	NC-12-007

Developmental and Reproductive Toxicology Studies:

Studies were not conducted to evaluate this safety endpoint based on the product type and due to the lack of findings in reproductive tissues in Study #10.

Genotoxicity Studies:

Study Number	Study Title / Publication Citation	Report Number
11	In Vitro Immortalization (IVIM) Assay Report	NC-12-008
12	Isolation of Cellular DNA from (b) (4) Mice Bone Marrow Engrafted with Lenti-D transduced Human CD34+ Hematopoietic Stem Cells from Study NC-12-007	NC-12-014
13	Integration Site Analyses of Lenti-D Transduced CD34+ Cells Following Xenotransplantation and Engraftment into (b) (4) Mice from Study NC-12-007	NC-12-009

Carcinogenicity Studies:

Traditional carcinogenicity studies were not conducted as they are not suitable for assessing this product type.

Note: Study Nos. 10, 11, and 13 are summarized in this review memo under ‘Overview of Toxicology Studies.’ Study #12 is not summarized as it describes the methods used to isolate and evaluate DNA integrity for the integration site analysis performed in Study #13.

Toxicology Studies**Study #10**

Report Number		NC-12-007-R
Date Report Signed		10-August-2012
Title		Single Dose Toxicity and Biodistribution Study of Lenti-D in Mice
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		The purpose of this study was to evaluate the potential toxicity and biodistribution of the test article, Lenti-D, after a single intravenous administration to male and female (b) (4) mice.
Study Animals	Strain/Breed	(b) (4)
	Species	Mouse
	Age	29-31 days old
	Body Weight	12.5-22.5 grams
	#/sex/group	12-15/sex/group for test and control article administered groups; 2/sex/group for untreated controls
	Total #	116
Test Article(s)		Lenti-D lentiviral vector transduced human CD34+ HSCs from healthy donors
Control Article(s)		Non-transduced human CD34+ HSCs from healthy donors
Route of Administration		Intravenous

Description of the Administration Procedure	All mice in Groups 1 and 2 received a subcutaneous dose of Busilvex® (30 mg/kg) on Day -1. Untreated control animals (Group 3) did not receive Busilvex® on Day -1. Animals were divided into three (3) sets. The day of dose administration for each set was designated Day 1 for that set. Each set of animals contained approximately the same number of animals from each group, sex, and sacrifice time, with the exception of untreated control animals, which were included in the first set. The animals in Groups 1 and 2 received the control or test article by intravenous bolus on Day 1. Each mouse received 1×10^6 cells/mouse. The vials containing the test and control articles (cell suspensions) were shaken gently before injection to ensure that cells did not settle.																																	
Study Groups and Dose Levels	<table border="1"> <thead> <tr> <th>Group</th><th>Number of Animals</th><th>Test Article</th><th>Dose (cells/animal)</th><th>Dose Volume (mL/animal)</th><th>Day 29 Interim Sacrifice</th><th>Day 92 Main Sacrifice</th></tr> </thead> <tbody> <tr> <td>1 Control</td><td>27M/27F</td><td>Nontransduced CD34+ HSCs</td><td>1×10^6</td><td>0.25</td><td>12M/12F</td><td>15M/15F</td></tr> <tr> <td>2 Treated</td><td>27M/27F</td><td>Lenti-D transduced CD34+ HSCs</td><td>1×10^6</td><td>0.25</td><td>12M/12F</td><td>15M/15F</td></tr> <tr> <td>Untreated Control</td><td>4M/4F</td><td>N/A</td><td>N/A</td><td>N/A</td><td>2M/2F</td><td>2M/2F</td></tr> </tbody> </table> <p>Source: Study Report NC-12-007-R, page 10 of 500</p>						Group	Number of Animals	Test Article	Dose (cells/animal)	Dose Volume (mL/animal)	Day 29 Interim Sacrifice	Day 92 Main Sacrifice	1 Control	27M/27F	Nontransduced CD34+ HSCs	1×10^6	0.25	12M/12F	15M/15F	2 Treated	27M/27F	Lenti-D transduced CD34+ HSCs	1×10^6	0.25	12M/12F	15M/15F	Untreated Control	4M/4F	N/A	N/A	N/A	2M/2F	2M/2F
Group	Number of Animals	Test Article	Dose (cells/animal)	Dose Volume (mL/animal)	Day 29 Interim Sacrifice	Day 92 Main Sacrifice																												
1 Control	27M/27F	Nontransduced CD34+ HSCs	1×10^6	0.25	12M/12F	15M/15F																												
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Untreated Control	4M/4F	N/A	N/A	N/A	2M/2F	2M/2F																												
Dosing Regimen	Single																																	
Randomization	Yes																																	
Description of Masking	Not provided in the study report																																	
Scheduled Sacrifice Time Points	Day 29 and Day 92 post administration																																	

Key Assessments:

- Clinical observations were performed once a day.
- Body weights were recorded once a week.
- Clinical pathology: Groups 1 and 2 animals were fasted overnight prior to blood collection. Blood samples from 6 interim sacrifice (day 29) and 8 main sacrifice (day 92) animals/sex/group were used for hematology parameter analysis. Blood samples from the remaining animals/sex/group were used for clinical chemistry assessment.
- Engraftment: FACS analysis was performed on bone marrow cells (femurs) for assessment of engraftment of human CD34+ cells using the human CD45, CD14, CD19, and CD33 markers.
- VCN/cell was assessed on blood and bone marrow samples collected at days 29 and 92.
- Necropsy and histopathology:
 - Gross necropsy examination and organ weights were performed for Groups 1 and 2 animals.
 - Tissue and organ collection: For paired organs, one was fixed in 10% neutral buffered formalin for histopathological evaluation, and the other was frozen for

DNA isolation (biodistribution analysis). For unpaired organs, one portion was frozen for DNA isolation and the other portion was fixed in 10% neutral buffered formalin for histopathological evaluation.

- Histopathology: Following fixation, the tissues from all Groups 1 and 2 animals were processed and histological slides of H&E-stained sections were prepared. A complete tissue set (including gross lesions) was evaluated by light microscopic examination. Histopathological tissue assessment was performed by a board-certified veterinary pathologist.
- Biodistribution was performed on blood and select tissues collected from 5 animals/sex/group at each time point.
- Bone marrow (tibias) samples were also collected for integration site analysis at each sacrifice time point (see Study #13).

Key Results:

Mortality: There were a total of 11 unscheduled deaths. Seven animals (five in Group 1 and two in Group 2) were found dead during the course of the study. Four animals (two each in Groups 1 and 2) were sacrificed in moribund condition with signs of swollen abdominal cavity, hunched posture, piloerection, and hydrocephalus.

Bone marrow cells were isolated from the femurs of six mice with unscheduled deaths across both groups and analyzed for human CD45+ and ALDP expression. Bone marrow samples from all 6 mice were 22-62% positive for CD45+, indicating human cell engraftment. Therefore, per the study report, lack of engraftment was not likely the reason for the early death in mice. In addition, the bone marrow from the mice that received Lenti-D transduced human CD34+ HSCs was positive for ALDP expression.

The tissues submitted for histopathological evaluation were too deteriorated to make any conclusion as to cause of death; however, given the higher incidence of unscheduled deaths in the control group, these deaths were not considered test article related by the pathologist.

Body weights: There were no test article related changes to body weights in this study.

Clinical observations: Abnormal clinical signs were observed through Day 29 and included hair loss (2/24 of Group 1M; 3/26 of Group 2F), wounds in the back or neck (1/24 of Group 1M; 3/26 of Group 2F), and piloerection (1/24 of Group 1F). No abnormal clinical signs were noted during the day 30-91 period.

During terminal clinical observations on Day 92, one Group 1 female appeared lethargic and hunched, and a Group 2 male was hydrocephalic. These observations were not considered test article-related.

Clinical pathology: There were no test article related abnormal changes in any clinical pathology parameter evaluated in this study.

Organ weights: On Day 29, a significant decrease in the mean absolute and relative spleen weights was observed in Group 2 males compared to controls. There was no histological correlate.

VCN/cell: In blood samples collected at Day 29 and Day 92 from test article administered mice, the VCN/cell ranged from 0.32-1.15 and 0.36-1.83, respectively. In bone marrow samples collected at Day 29 and Day 92 from test article administered mice, the VCN/cell ranged from 1.01-2.64 and 0.48-2.13, respectively.

Gross pathology: There were no test article related abnormal findings on gross pathology in this study.

Histopathology: There were no test article related abnormal findings on histopathology in this study.

Biodistribution: For the detection of human cells and viral vectors, (b) (4) were used, respectively. (b) (4) was detected in all the tissues analyzed from Groups 1 and 2 animals. The tissue showing the highest number of copies was the bone marrow. (b) (4) was detected in all the analyzed tissues/organs of Group 2 animals only (test article administered). In both males and females, the maximal concentration of (b) (4) at the analyzed time points was observed in bone marrow (12790-235419 average numbers of copies per microgram of DNA).

Conclusion: Although there was significant mortality in this study, the distribution was similar between control and test article administered groups. Therefore, these deaths were not attributed to the test article. Administration of the test article appeared generally well tolerated in this study when administered IV to mice at a dose level of 1×10^6 CD34+ cells/mouse or approximately 5×10^7 CD34+ cells/kg (based on a 20-gram mouse).

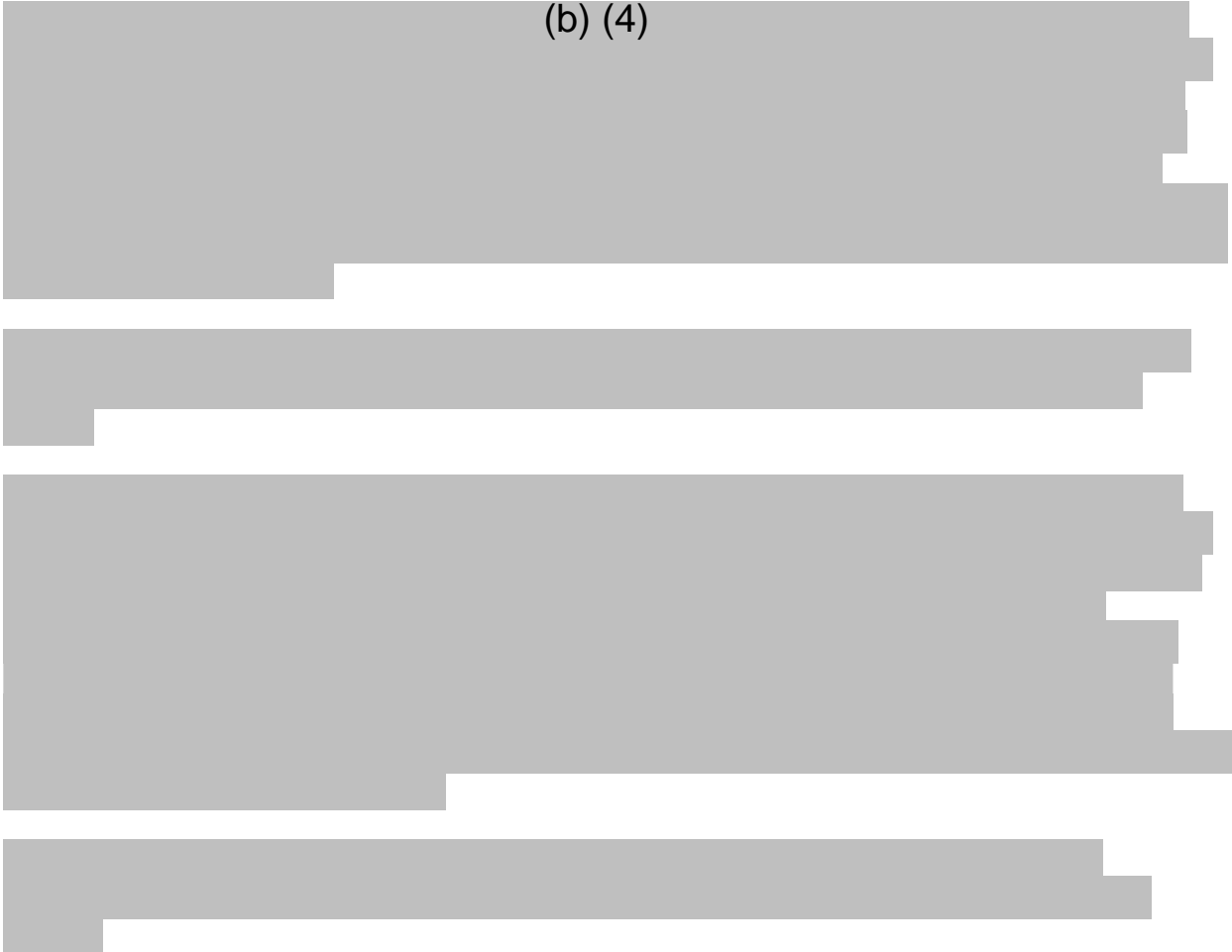
Reviewer Comments:

- This study utilized myeloablation prior to administration of the both the test and control articles. It is possible that the myeloablation regimen in young (b) (4) mice contributed to the early mortality in this study as no deaths were observed in the Group 3, untreated control animals.
- The dose level administered in this study was the maximum feasible dose level as determined by the applicant. Although a no observed effect level (NOAEL) could not be determined, a dose level of 1×10^6 CD34+ cells/animal or 5×10^7 CD34+ cells/kg provides an approximately 10-fold safety margin to the recommended minimum dose level to be administered in humans (5×10^6 CD34+ cells/kg).

Study #11

(b) (4)

(b) (4)



Study #13

Objective: The objective of this study was to evaluate the genomic DNA (gDNA) integration site analysis (ISA) profiles of pre-transplant Lenti-D transduced mPB-derived CD34+ HSCs and post-transplant Lenti-D transduced bone marrow cells (BMCs) collected from the pivotal toxicology study in myeloablated immunodeficient (b) (4) mice (Study #10).

Study Design: The test articles for this study were gDNA samples from pre-transplant Lenti-D transduced human healthy donor CD34+ HSCs and Month 3 post-transplant Lenti-D transduced BMCs from tibias of myeloablated (b) (4) mice. Samples were prepared and selected from animals with sufficient Lenti-D transduction levels ($\text{VCN} \geq 0.88$ c/dg and DNA >100 ng) to facilitate ISA. The control articles were gDNA samples from pre-transplant mock-transduced human healthy donor CD34+ HSCs and Month 3 post-transplant mock-transduced BMCs from tibias of myeloablated (b) (4) mice. ISA profiling for all gDNA samples included LAM-PCR, pyrosequencing for integration sites (IS) and common integration site (CIS) analysis.

Results: LAM-PCR analysis of ex vivo transduced CD34+ HSCs revealed a polyclonal pattern, while an oligo- to polyclonal pattern was observed in the bone marrow of (b) (4) mice. No obvious

difference was observed in the clonal size of bone marrow cells harvested at 30 days and 90 days, based on (b) (4) from LAM-PCR amplicons, and sequencing data.

The integration profile of Lenti-D vector in human CD34+ HSCs was characterized and the occurrence and frequency of CIS was also determined. A preferred integration of Lenti-D vector was observed on chromosomes 17 and 19. A strong enrichment of Lenti-D IS within gene coding regions and the 10 kb surrounding region was observed. In contrast to conventional gamma-retroviral vectors, a preference of Lenti-D integration for transcriptional start sites (± 10 kb) was not observed. No obvious differences in integration profiles were observed between samples harvested at 30- or 90-days post transplantation.

In previous studies, CIS have demonstrated potential as an indicator for clonal skewing.(11) The following definition for CIS determination was used, 2nd order CIS: 2 IS in 30 kb; 3rd order CIS: 3 IS in 50 kb; 4th order CIS: 4 IS in 100 kb; $\geq 5^{\text{th}}$ order CIS: 5 or more IS in 200 kb. The highest orders of CIS observed were a single 7th order CIS, six CIS of 6th order, three CIS of 5th order, and six CIS of 4th order. CIS of 4th and higher order have been previously identified in a preclinical study of MLD(12) and in a lentiviral clinical ALD trial.(13)

Conclusions: In this study, Lenti-D demonstrated a similar SIN-lentiviral integration profile for dividing cells compared to published studies.(13, 14) This included preferred integration in gene coding regions and no preference for transcriptional start sites. Integration within genes occurred over the whole gene coding region, with no bias for the 5' or 3' end of genes. Additionally, the Lenti-D vector showed a modest frequency (19.04%) of integration sites forming CIS, with one CIS of 7th order being the largest cluster followed by six CIS of 6th order.

Reviewer Comments:

- *This nonclinical study did not show occurrence of vector integration within the MECOM gene that has been identified in multiple clinical trial subjects who have developed myelodysplastic syndrome (MDS). However, there were several differences between this study and the clinical situation.*
 - *The cells used in this study were from healthy human donors, not CALD patients. The differences between healthy and CALD patient cells may affect vector integration.*
 - *The longest evaluation time point in this study was 90 days. This may not be of sufficient length to detect such integration events as the events identified in the clinical trial occurred at much later follow up time points (up to 7 years later).*

APPLICANT'S PROPOSED LABEL

Subsections 8.1 of Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14).

Section 13.2 ('Animal Toxicology and/or Pharmacology') should be revised to include only information from the nonclinical studies necessary for the safe and effective use of the product.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns for the clinical dosing, route of administration, and indications for use. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

SKYSONA, eli-cel, lentiviral vector, hematopoietic stem cells, adrenoleukodystrophy

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