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I concur with this review memo. A. Wensky. 1/27/2026

I concur with this review memo. D. Brooks 1/27/2026

**FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Therapeutic Products
Office of Pharmacology/Toxicology
Division of Pharmacology/Toxicology 1
Pharmacology/Toxicology Branch 3**

BLA NUMBER: 125806	STN #125806.000
DATE RECEIVED BY CBER ⁱ :	BLA resubmitted on September 26, 2025 A CR letter was previously sent on June 14, 2024, but were not related to any P/T review issues. There were no changes to the P/T review memo other than updated dates, reviewers, and minimal formatting changes.
DATE REVIEW COMPLETED:	January 20, 2025
PRODUCT ⁱⁱ :	KRESLADI (RP-L201, marnetegrane autoemcel) suspension for intravenous infusion
APPLICANT:	Rocket Pharmaceuticals, Inc.
PROPOSED INDICATION ⁱⁱⁱ :	For the treatment of severe Leukocyte Adhesion Deficiency-I (LAD-1)
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EXECUTIVE SUMMARY⁴:

KRESLADITM (Marnetegrane autoemcel or RP-L201) consists of autologous CD34+ hematopoietic stem cells (HSCs) transduced with a self-inactivating lentiviral vector (SIN-LV) encoding the therapeutic integrin subunit beta 2 (ITGB2 or CD18) gene for treatment of pediatric patients with leukocyte adhesion deficiency-I (LAD-I). The LAD-I indication includes (b) (4)

(b) (4) the severe phenotype expressing (b) (4) of CD18. Deficiencies in CD18 prevent normal dimerization with their corresponding α -subunits (α L-CD11a; α M-CD11b; α X-CD11c and α D-CD11d), impairing neutrophils ability to adhere firmly to inflamed endothelium, which is needed for extravasation to infected and/or inflamed tissues. Following intravenous (IV) infusion in the patient, KRESLADI™ is intended to engraft in the bone marrow (BM) and differentiate to produce neutrophils expressing biologically active CD18 that will result in dimerization to the corresponding CD11 α -subunits and correction of neutrophil function.

In vitro pharmacology studies were conducted using an immortalized LAD-I patient-derived lymphoblastic cell line (LCL) as well as healthy donor (HD) cord blood-derived (CB) CD34+ HSCs transduced with short hairpin ribonucleic acid (shRNA) to knock down CD18 expression and evaluate the activity of the Chim-CD18-WPRE LV used in RP-L201. These studies demonstrated that vector-driven ITGB2 transgene expression led to hCD18:CD11a receptor dimerization, improved neutrophil aggregation, intercellular adhesion molecule-I (ICAM-I) binding, resistance to shear stress, and respiratory burst function.

In vivo studies were conducted in either CD18 hypomorphic (CD18^{HYP}) or CD18 knockout (CD18^{KO}) mice to assess the safety and activity of lineage-negative (Lin-) murine hematopoietic stem and progenitor cells (mHSPCs) transduced with the Chim-CD18-WPRE LV used in RP-L201. CD18^{HYP} and CD18^{KO} mice have deficient CD18 expression and impaired neutrophil migration and inflammatory responses similar to LAD-I patients. Successful engraftment of the Chim-CD18-WPRE LV transduced Lin- cells was demonstrated in recipient CD18^{HYP} or CD18^{KO} mice with enhanced neutrophil extravasation to tissue-specific sites of inflammation. Likewise, bone marrow cells (BMCs) isolated from CD18^{HYP} mice were re-transplanted into secondary recipients and showed stable vector copy number (VCN), multilineage reconstitution, and increased human CD18 (hCD18) expression. The transduction enhancers (TEs) (b) (4) augmented hCD18 expression and improved neutrophil extravasation and function in both murine LAD-I models.

A 1-month in vivo toxicology and biodistribution (BD) study was also conducted using recipient (b) (4) mice for safety evaluation of Chim-CD18-WPRE LV transduced Lin- murine HSPCs. No premature mortality or unexpected deaths were observed over the course of the study period in recipient mice. Hematopoietic reconstitution was observed in the peripheral blood (PB) and BM and measurable VCN was detected in other hematopoietic organs, whereas undetectable or low VCN was observed in non-hematopoietic organs including the gonads.

In vivo studies using HD mobilized peripheral blood (mPB)-, or CB-derived CD34+ HSCs with or without TEs administered to immunodeficient NOD.Cg-(b) (4)^{scid}IL2rg^{(b) (4)}/SzJ (NSG) mice demonstrated successful hematopoietic lineage reconstitution and hCD18 expression following transduction with the Chim-CD18-WPRE LV as compared to the untransduced (UNT) and (b) (4) controls. Human CD45+ cells isolated from the BM of primary recipients were re-transplanted into secondary recipients and showed multilineage hematopoietic reconstitution and sustained hCD18 expression.

Insertional analysis was conducted using PB and BM of CD18^{HYP} mice transplanted with Lin-murine HSPCs transduced with Chim-CD18-WPRE LV from both primary and secondary recipients for different post-transplantation timepoints (1, 4, or 9 months). The insertion sites (ISs) of transduced cells and primary transplanted samples in mice showed an oligoclonal to polyclonal vector integration profile indicating no clonality. A decrease in clonal diversity from primary to secondary recipients with multiple identical clones contributing to hematopoietic repopulation was observed as expected for the secondary transplantation studies. There were no preferential integrations in or nearby genes previously associated with insertional mutagenesis. Similarly, NSG mice transplanted with HD mPB and CB CD34⁺ cells transduced with Chim-CD18-WPRE LV showed a polyclonal integration profile.

Carcinogenicity and developmental and reproductive toxicity studies were not conducted with KRESLADITM. These studies are not warranted based on the drug product characteristics and safety profile.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION⁵:

There are no nonclinical deficiencies in the pharmacology/toxicology studies. There are no requests for further nonclinical testing of KRESLADITM. The nonclinical data provided in this BLA submission support the approval of the licensure application.

Formulation and Chemistry⁶:

Marnetegrage autoemcel (i.e., RP-L201) is a cell suspension for IV infusion. Its active drug substance consists of autologous CD34⁺ HSCs from LAD-I patients transduced with the SIN-LV encoding the human ITGB2 transgene complementary DNA (cDNA) that expresses CD18. A single dose of RP-L201 contains a minimum recommended dose of 1×10^6 CD34⁺ cells/kg of body weight, suspended in a solution containing 5% dimethyl sulfoxide (DMSO).

Abbreviations

PMA	4-beta-phorbol-12-myristate-13-acetate
AP	Air pouch
BD	Biodistribution
BM	Bone marrow
CD3	Cluster of differentiation 3
CD11a	Cluster of differentiation 11a
CD18 ^{HYP}	Cluster of differentiation 18 hypomorphic
CD18 ^{KO}	Cluster of differentiation 18 knockout
CD34	Cluster of differentiation 34
CD45R	Cluster of differentiation 45R
CIS	Common Integration Site
CB	Cord Blood
CCND2	Cyclin D2
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide

DP	Drug product
EVI-1	Ecotropic virus integration site 1 protein homolog
(b) (4)	
(b) (4)	
FBXO8	F-Box protein Fbx8
FDA	Food and Drug Administration
gDNA	Genomic Deoxyribonucleic acid
Gm156	Killer cell lectin-like receptor subfamily H, member 1
GMP	Good Manufacturing Practice
GvHD	Graft-versus-host-disease
Gy	Gray
HD	Healthy donor
HSC	Hematopoietic stem cell
HSPC	Hematopoietic stem and progenitor cells
HSCT	Hematopoietic stem cell transplant
HVCN	High vector copy number
hCD18	Human Cluster of differentiation 18
HIV	Human Immunodeficiency Virus
IU	Infectious Units
IS	Integration Site
ISA	Integration Site Analyses
IV	Intravenous
(b) (4)	
LV	Lentiviral Vector
LAD-I	Leukocyte Adhesion Deficiency-I
LMO2	LIM Domain Only 2
Lin-	Lineage negative
LSK	Lin-Sca1+cKit+
LPS	Lipopolysaccharide
LT-HSCs	Long-Term Hematopoietic stem cells
LVCN	Low vector copy number
LCL	Lymphoblastoid cell lines
Ly6G	Lymphocyte antigen 6 complex locus G6D
LFA-1	Lymphocyte function associated antigen-1
Mac-1	Macrophage-1 antigen
MECOM	MDS1 and EVI-1 Complex Locus
MFI	Mean fluorescence intensity
mRNA	Messenger ribonucleic acid
mPB	Mobilized peripheral blood
MN1	MN1 Proto-Oncogene, Transcriptional Regulator
MOI	Multiplicity of infection
MDS1	Myelodysplasia syndrome 1
MDS	Myelodysplastic syndrome
NSG	NOD.Cg-(b) (4) ^{scid} IL2rg ^{(b) (4)} /SzJ

(nr)LAM-PCR	Non-restrictive Linear-amplification mediated polymerase chain reaction
NUDT3	Nudix Hydrolase 3
O.N.	Overnight
PB	Peripheral blood
PBL	Peripheral blood leukocytes
PBS	Phosphate buffered saline
(b) (4)	
PCR	Polymerase chain reaction
PID	Primary immunodeficiency
POC	Proof-of-concept
(b) (4)	
RCL	Replication competent lentivirus
RUNX1	Runt-related transcription factor 1
RP-L201	Marnetegrane autoemcel
SAE	Serious adverse event
SIN	Self-inactivating
SETD4	SET Domain Containing 4
S-EPTS/LM-PCR	Shearing extension primer tag selection/ligation-mediated-polymerase chain reaction
shRNA	Short hairpin ribonucleic acid
sICAM-1	Soluble intercellular adhesion molecule-1
(b) (4)	
STAG2	STAG2 Cohesion Complex Component
Sca-1	Stem cell antigen-1
SC	Subcutaneous
TE	Transduction Enhancer
TSS	Transcription start site
TNF- α	Tumor necrosis factor- α
(b) (4)	
UNT	Untransduced
VCN	Vector copy number
WBC	White blood cell
WT	Wild-type
WPRES	Woodchuck hepatitis post-transcriptional regulatory element
YLP1	YLP Motif Containing 1

Related File(s):

- **IND#18485:** Hematopoietic stem cells modified with a lentiviral vector (Chim.hCD18-LV) encoding for the human Beta 2 Integrin/CD18 gene [RP-L201]; Treatment of Severe Leukocyte Adhesion Deficiency-I (LAD-I); Rocket Pharmaceuticals, Inc.

Cross-referenced File(s):

- MF (b) (4) [MASTER FILE TYPE V: (b) (4) manufacturing facility; manufacture of product; (b) (4)]

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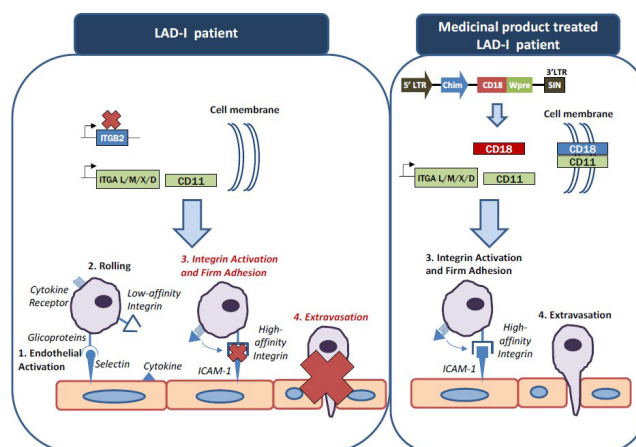
INTRODUCTION

LAD-I is an autosomal recessive primary immunodeficiency (PID), with an estimated global prevalence of 1.2 in 1,000,000. LAD-I is characterized by recurrent and severe bacterial and fungal infections, which are associated with mutations in the ITGB2 gene that encode for the common β -subunit (CD18) of the β_2 integrin family of receptors (M Madkaikar 2012). The β -subunit heterodimerizes with 4 different α -subunits (α L-CD11a; α M-CD11b; α X-CD11c; α D-CD11d) that function as leukocyte receptors for intercellular adhesion molecules. Deficiencies in CD18 prevent integrin dimerization and result in impaired neutrophil adhesion to endothelial surfaces, which is needed for leukocyte extravasation to stop local tissue infections (Yu-chen Gu 2004).

Currently, there is no FDA-approved treatment for LAD-I. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only known curative treatment for LAD-I. However, allogeneic HSCT for treatment of LAD-I has been associated with considerable rates of severe and life-threatening toxicities including chronic graft versus host disease (GvHD) as well as bacterial, viral, and fungal infections arising from transplant-related immunosuppression (Shahzard Bakhtiar 2021). RP-L201 is an autologous HSC product that is transduced with a LV encoding

the ITGB2 complementary DNA (cDNA) for expression of CD18 in neutrophils. After RP-L201 is infused, transduced CD34⁺ HSCs engraft in the bone marrow and subsequently differentiate into various cells, including neutrophils that express sufficient levels of CD18/CD11a to interact with endothelial intercellular adhesion molecule-1 (ICAM-1) needed for tissue-specific extravasation to sites of infection (Figure 1).

Figure 1. Expression of the Chim-hCD18-WPRE LV in CD34⁺ HSCs Induces Genetic correction of Leukocytes.



NONCLINICAL STUDIES

Products evaluated in the nonclinical studies

The final clinical product and source material was not used in the nonclinical studies summarized in this memo due to feasibility issues obtaining sufficient CD34⁺ HSCs from LAD-I patients. Instead, the applicant primarily used immortalized LAD-I LCL cells and HD CB CD34⁺ HSCs modified with shRNA targeting mRNA transcripts to knock down CD18 expression for their in vitro pharmacology studies. The applicant also used surrogate Lin⁻ HSPCs from CD18^{HYP} and CD18^{KO} mice transduced with the clinical vector to demonstrate donor cell engraftment and improved neutrophil extravasation to tissue-specific sites of inflammation for the in vivo pharmacology studies.

Due to the lack of available LAD-I patient CD34⁺ HSCs, the applicant similarly used HD CB- or PB-derived CD34⁺ HSCs (with normal CD18 expression) transduced with the Chim-CD18-WPRE LV transplanted into NSG mice to assess the in vivo engraftment of human HSPCs. The applicant also used surrogate Lin⁻ HSPCs transduced with the Chim-CD18-WPRE LV transplanted into recipient (b) (4) mice for their definitive in vivo toxicology and BD studies.

All cells were transduced with the clinical vector, Chim-CD18-WPRE LV, which is used to produce RP-L201. The LV contains a chimeric 'chim' promoter, which is active in macrophages and neutrophils. The 'chim' promoter elicits myeloid-biased expression both in human cell lines and in mice. A mutated woodchuck hepatitis post-transcription regulatory element (WPRE)

lacking any residual open reading frame was included in all evaluated vectors to stabilize hCD18 expression and improve the long-term construct expression in vivo (A Schambach 2006).

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies⁷

The following pharmacology studies were conducted to support the rationale for the administration of RP-L201 to treat the proposed clinical indication.

In Vitro Studies

Study Number	Study Title / Publication Citation	Report Number
1	Development of the Chim-CD18-WPRE LV as an efficient vector able to phenotypically correct an LAD-I patient-derived lymphoblast cell line	NSR-1
2	Assessment of the ability of Chim-CD18-WPRE LV to induce in vitro phenotypic correction in LAD-I-like human primary hematopoietic cells	NSR-2

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
3	Assessment of the ability of the Chim-CD18-WPRE LV to phenotypically correct LAD-I-like features in a mouse model harboring a CD18 hypomorphic mutation	NSR-3
4	Assessment of in vivo engraftment of human hematopoietic progenitors transduced with Chim-CD18-WPRE lentiviral vector in NSG mice	NSR-4
5	Assessment of the use of transduction enhancers (b) (4) to improve Chim-CD18-WPRE transduction efficiency of CD34+ cells and maintain the reconstitution potential of the transduced cells	NSR-5
6	Assessment of the use of transduction enhancers (b) (4) to improve Chim-CD18-WPRE LV transduction efficiency and in vivo phenotypic correction in LAD-I mouse models	NSR-6

Mouse models/strains:

- The CD18^{HYP} mouse model contains the Itgb2^{tm1Bay} allele, which is a hypomorphic insertion mutation rather than a null allele that causes reduced expression levels of CD18. Homozygous CD18^{HYP} mice are viable and fertile and exhibit mild myeloid hyperplasia of the BM and splenic red pulp, neutrophilia, high numbers of white blood cells (WBCs), and granulocytosis. The CD18^{HYP} mice were used as a model for human CD18 deficiency and display similar phenotypic characteristics observed in LAD-I patients including reduced CD18 expression, reduced neutrophil extravasation, and increased susceptibility to opportunistic infections (Raymond W. Wilson, 1993).
- The null CD18^{KO} mouse more closely mimics the LAD-I phenotype with impaired neutrophil extravasation, marked neutrophilia, splenomegaly, chronic dermatitis, spontaneous cutaneous infections, and severe defects in T cell proliferation. CD18^{KO}

mice also have difficulties in breeding and colony maintenance (Karin Scharffetter-Kochanek, 1998). CD18^{KO} mice also display an increase in Lin-Sca1+cKit+ (LSK) cells in the BM and delayed apoptosis of circulating neutrophils as compared to wild-type (WT) mice (John C. Gomez, 2010).

- NSG (i.e., NOD.Cg-(b) (4)^{scid}IL2rg^{(b) (4)}/SzJ) mice are an immunodeficient mouse strain that are permissive to human CD34+ HSC engraftment and permit the study of hematopoietic reconstitution and lymphoid development in vivo.
- C57BL/6J mice were used as the CD45.2 genetic background inbred CD18^{WT} control strain for the studies utilizing the CD18^{HYP} and CD18^{KO} LAD-I-like mouse models. Hybrid mice were also generated as the Lin- donors derived from the crossed (b) (4) mice into recipient crossed (b) (4) mice for toxicological and BD evaluation of the mouse surrogate HSCs transduced by the Chim-CD18-WPRE LV. (b) (4) mice are a (b) (4) congenic strain that is widely used in transplant studies because it carries the differential *Ptprc*^a pan leukocyte marker commonly known as (b) (4). The WT (b) (4) inbred mice express the *Ptprc*^b (CD45.2 or Ly5.2) allele.

Overview of Pharmacology Studies

Overview of In Vitro Studies

Study #1

Study objective: This study was conducted to evaluate the transduction efficiency in human LAD-I-deficient LCL cells transduced with the following LV vectors containing different internal promoters: 1) Chim-hCD18-WPRE LV; (b) (4).

Study design: The LAD-I LCL cell line was transduced with (b) (4) different LVs (i.e., ‘Chim’-hCD18-WPRE LV; (b) (4)) at different multiplicities of infection (MOI) ranging from 1 to 100 infectious units (IU)/cell. Cells were harvested after 10 – 14 days of culture to measure: 1) VCN; 2) CD18/CD11a expression; 3) 4-beta Phorbol 12-myristate 13-acetate (PMA) induced aggregation; 4) sICAM-1 binding.

Results: All (b) (4) LVs (i.e., Chim-CD18-WPRE LV; (b) (4)) resulted in similar restoration of CD18/CD11a membrane expression in transduced LAD-I LCL cells. Despite increasing MOI of Chim-CD18-WPRE LV, membrane expression for both CD18 and CD11a reached a plateau at a MOI of 10 IU/cell. Likewise, all (b) (4) LVs restored PMA-stimulated aggregation and sICAM-1 binding in transduced LAD-I LCL cells.

Reviewer Comment:

- The endogenous expression of CD11a limits the maximum amount of CD18 expressed in the membrane, because CD18/CD11a dimerization is needed for sustained cell surface expression. The myeloid-biased expression of genes under the control of the ‘Chim’

promoter places limitations on CD18 expression as an additional safety feature for gene therapy in LAD-I patients as compared to the ubiquitously expressed (b) (4) promoters.

Study #2

Study objective: To demonstrate that transduction with the Chim-CD18-WPRE LV restores the functional properties of LAD-I-like neutrophils with evaluation of: 1) CD18/CD11a expression; 2) sICAM-1 binding; 3) resistance to shear forces; 4) respiratory burst assay.

Study design: HD CB-derived CD34+ HSCs were used to generate LAD-I-like CD34+ HSCs by transducing with shRNA targeting CD18 mRNA at a MOI of 100 IU/cell. After 3 days, the LAD-I-like CD34+ HSCs were subsequently transduced with Chim-CD18-WPRE LV at a MOI of 100 IU/cell. The CD34+ HSCs were then cultured in neutrophil differentiation medium and allowed to proliferate for 12 days before conducting the neutrophil assays.

Results: The LAD-I-like neutrophils derived from Chim-CD18-WPRE LV transduced CD34+ HSCs had a 43% recovery of hCD18 levels with similar trends in human CD11a/CD11b expression relative to the control neutrophil hCD18 levels from HDs. The LAD-I-like Chim-CD18-WPRE-LV transduced neutrophils also showed intermediate ability to bind sICAM-1, resistance to shear stress, and respiratory burst function that was greater than the LAD-I-like negative control but less than the HD-derived positive control.

Overview of In Vivo Studies

In Vivo Studies in Healthy Animals

Study #3

Report Number	NSR-3
Date Report Signed	03-May-2023
Title	Assessment of the ability of the Chim-CD18-WPRE LV to phenotypically correct LAD-I-like features in a mouse model harboring a CD18 hypomorphic mutation
GLP Status	No
Testing Facility	(b) (4)
Objective(s)	<ol style="list-style-type: none"> 1. To evaluate the potential therapeutic efficacy of (b) (4) LVs expressing CD18 (Chim-CD18-WPRE LV; (b) (4)) to efficiently transduce HSCs, restore CD18 expression, and restore neutrophil function in a mouse model of LAD-I with a hypomorphic mutation in CD18. 2. To enable analysis of the safety of the Chim-CD18-WPRE LV by investigating the pattern of LV insertion sites and the in vivo clonal dynamics of cells from CD18^{HYP} mice transduced with Chim-CD18-WPRE LV.

Strain/Breed	1. B6.129S7-Itgb2 ^{tm1Bay} /J CD18 ^{HYP} (CD18 ^{HYP} mice) 2. C57BL/6J B6 wild-type mice
Species	<i>Mus musculus</i>
Age	8 - 12 weeks
Body Weight	~20 g Note: The sponsor did not provide initial body weight data for this study. The average body weight for this species was used instead.
#/sex/group	See the study group and dose levels tables below for specific animal numbers per group which vary in terms of MOI (IU/cell), donor cells (CD18 ^{HYP} or CD18 ^{WT}), LVs (Chim-CD18-WPRE LV, (b) (4), and the number of primary and secondary recipients. The exact number of males and females was not specified by the applicant.
Total #	The primary transplant cohort included 102 mice and the secondary transplant cohort included 16 mice. Of the primary transplant recipients, 45 mice received cells transduced with Chim-hCD18-WPRE LV, 35 mice received cells transduced with (b) (4), and 22 mice received cells transduced with (b) (4). For the secondary recipients, 7 mice received cells transduced with the Chim-CD18-WPRE LV, 4 mice received cells transduced with the (b) (4), and 5 mice received cells transduced with the (b) (4). Likewise, mice transplanted with (b) (4)-transduced CD18 ^{WT} Lin- BM cells served as the positive control, while 25 CD18 ^{HYP} mice transplanted with (b) (4)-transduced CD18 ^{HYP} Lin- BM cells served as the negative control.
Test Article(s)	1. Chim-hCD18-WPRE LV (b) (4)
Control Article(s)	(b) (4)
Route of Administration	IV administration
Description of the Animal Model and Dose Levels	<u>Ex Vivo Gene Therapy Studies</u> Lin- cells enriched for mouse HSPCs were transduced with (b) (4) hCD18-LVs (Chim-CD18-WPRE LV, (b) (4)) at a MOI of 20 – 50 IU/cell. The transduced cells (3 – 5 x 10 ⁵ cells/animal) were transplanted into CD18 ^{HYP} mice that were irradiated the day prior to and on the day of transplantation (4.75 Gy + 4.75 Gy). At 4 months, primary CD18 ^{HYP} mice that received the Lin- transduced cells were euthanized and PB and BM was collected. The BM was similarly transplanted (3 x 10 ⁶ cells/animal) into lethally irradiated CD18 ^{HYP} secondary mice.
Dosing Regimen	Single administration
Randomization	Yes
Description of Masking	No
Scheduled Sacrifice Time Points	Primary mice were sacrificed at 4 months and secondary mice were sacrificed at 9 months post-transplantation.

Study Groups and Dose Levels:

Table 1. Ex vivo gene therapy in CD18^{HYP} Lethally Irradiated Animals

Study	Donor Cells	Vector	No. of Primary Recipients	No. of Secondary Recipients
1* ^{§†}	CD18 ^{HYP}	Chim-CD18-WPRE LV	3	4
1* ^{§†}	CD18 ^{HYP}	(b) (4)	4	5
2* ^{§†}	CD18 ^{HYP}	Chim-CD18-WPRE LV	3	3
2* ^{§†}	CD18 ^{HYP}	(b) (4)	5	4
2* ^{§†}	CD18 ^{HYP}	(b) (4)	5	-
2* ^{§†}	CD18 ^{WT}	(b) (4)	3	-
3 [†]	CD18 ^{HYP}	Chim-CD18-WPRE LV	5	-
3 [†]	CD18 ^{HYP}	(b) (4)	6	-
3 [†]	CD18 ^{HYP}	(b) (4)	4	-
3 [†]	CD18 ^{HYP}	(b) (4)	4	-
3 [†]	CD18 ^{WT}	(b) (4)	4	-
4 [‡]	CD18 ^{HYP}	Chim-CD18-WPRE LV	3	-
4 [‡]	CD18 ^{HYP}	(b) (4)	4	-
4 [‡]	CD18 ^{HYP}	(b) (4)	4	-
4 [‡]	CD18 ^{HYP}	(b) (4)	3	-
4 [‡]	CD18 ^{WT}	(b) (4)	2	-
5 [‡]	CD18 ^{HYP}	Chim-CD18-WPRE LV	7	-
5 [‡]	CD18 ^{HYP}	(b) (4)	5	-
5 [‡]	CD18 ^{HYP}	(b) (4)	4	-
5 [‡]	CD18 ^{WT}	(b) (4)	6	-
6 ^{&‡}	CD18 ^{HYP}	Chim-CD18-WPRE LV	8	-
6 ^{&‡}	CD18 ^{HYP}	(b) (4)	2	-
6 ^{&‡}	CD18 ^{HYP}	(b) (4)	3	-

6 ^{&‡}	CD18 ^{HYP}	(b) (4)	4	-
6 ^{&‡}	CD18 ^{WT}	(b) (4)	3	-
7 [‡]	CD18 ^{HYP}	Chim-CD18-WPRE LV	5	-
7 [‡]	CD18 ^{HYP}	(b) (4)	6	-
7 [‡]	CD18 ^{HYP}	(b) (4)	4	-
7 [‡]	CD18 ^{WT}	(b) (4)	2	-
8 ^{#‡}	CD18 ^{HYP}	Chim-CD18-WPRE LV	6	-
8 ^{#‡}	CD18 ^{HYP}	(b) (4)	2	-
8 ^{#‡}	CD18 ^{HYP}	(b) (4)	2	-
8 ^{#‡}	CD18 ^{WT}	(b) (4)	2	-
9 ^{#‡}	CD18 ^{HYP}	Chim-CD18-WPRE LV	5	-
9 ^{#‡}	CD18 ^{HYP}	(b) (4)	5	-
9 ^{#‡}	CD18 ^{HYP}	(b) (4)	3	-
9 ^{#‡}	CD18 ^{HYP}	(b) (4)	3	-
9 ^{#‡}	CD18 ^{WT}	(b) (4)	4	-

* In studies 1 and 2, secondary transplants were also performed.

[§]ISA (b) (4) analyses were performed in Chim-CD18-WPRE LV primary and secondary recipients from studies 1 and 2.

[&]Air pouch (AP) tumor necrosis factor- α (TNF- α) inflammation assay was performed in some of these animals.

[#]Lipopolysaccharide (LPS)-induced asthma assay was performed in some of these animals.

[†]In studies 1 – 3 an MOI (IU/cell) of 20 was used to transduce Lin- BM cells.

[‡] In studies 4 – 9 an MOI (IU/cell) of 50 was used to transduce Lin- BM cells.

Table 1 includes the total numbers of animals in each of the 9 individual studies conducted. The primary transplant cohort included 102 mice and the secondary transplant cohort included 16 mice. Of the 102 primary recipients, 45 mice received Lin- BM cells transduced with Chim-hCD18-WPRE LV, 35 mice received Lin- BM cells transduced with (b) (4), and 22 mice received Lin- BM cells transduced with (b) (4).

Mice transplanted with gene-corrected cells were compared with the following positive and negative control mice:

- Positive control: 26 CD18^{WT} mice transplanted with (b) (4)-transduced CD18^{WT} Lin- BM cells

- Negative control: 25 CD18^{HYP} mice transplanted with (b) (4) -transduced CD18^{HYP} Lin- BM cells

Key Evaluations and Assessments⁸:

- Lethally irradiated CD18^{HYP} primary mice that received Lin- transduced cells were monitored for survival as well as any gross physical, behavioral, or morphological abnormalities for 4 months. Blood was collected from recipients at 30-, 60-, 90-, and 120-days post-transplant.
- Lethally irradiated CD18^{HYP} secondary mice that received BMCs from CD18^{HYP} primary mice were similarly followed for 9 months. Blood was collected from recipients at 30, 60, 90, 120, 150 180, 210-, 240-, and 270-days post-transplantation.
- Collected PB was analyzed by flow cytometry for the expression of human CD18 in different leukocyte populations (i.e., T cells, B cells, and granulocytes using the corresponding markers CD3, CD45R, and Gr1, respectively) as well as the corresponding murine CD11a marker in murine neutrophils.
- *AP TNF- α Inflammation Model:* The AP was generated by dorsal subcutaneous (SC) injection of 5 mL of air on Day 0 under isoflurane anesthesia. On Day 3, the pouches were re-inflated with 3 mL of air and on Day 5, 40 ng of mouse recombinant TNF- α in phosphate buffered saline (PBS) with 0.5% carboxymethylcellulose inert carrier were injected into mature pouches. The PB and SC APs were collected for evaluation of neutrophil extravasation ratios for the (b) (4) LVs as compared to the control (b) (4) or to CD18^{WT} mice. The extravasation ratio for total leukocytes or neutrophils was calculated with the following equation:

$$\text{Extravasation Ratio} = \frac{\text{CCCCCCCCC iiiii tthCC AAAA}}{\text{CCCCCCCCC iiiii tthCC AAPP}} \times 100$$

- *LPS-Induced Asthma Model:* Mice were administered 50 μ L of 0.3 μ g/ μ L LPS [from *Escherichia coli* (b) (4)] or PBS intranasally under isoflurane anesthesia. Twenty-four hours after LPS/PBS administration, mice were euthanized and bronchoalveolar lavages (BALs) were immediately collected and compared to the control (b) (4) or to CD18^{WT} mice. The absolute number of neutrophils was determined using the following equation:

$$\text{Absolute Number of Neutrophils} = \text{Total Cell Number} * (\% \text{ Ly6G}^{\text{High}} \text{CD11c}^+ \text{ cell})$$

- The extravasation ratio for total leukocyte BALs and SC APs was determined for the (b) (4) LVs as compared to the Lin- cells derived from CD18^{HYP} (negative control) or CD18^{WT} mice (positive control) transduced with (b) (4) in recipient CD18^{HYP} and CD18^{WT} mice.
- VCN was determined and genomic DNA (gDNA) was isolated for ISA that was analyzed in Study #8 (NSR-8).

Key Results:

In vitro Chim-CD18-WPRE LV Transduction of Mouse HSPCs: Lin- BM cells from CD18^{HYP} mice were transduced with the (b) (4) different hCD18-LVs and differentiated into the myeloid lineage in vitro. Approximately 30 - 40% cell surface expression of hCD18⁺ was observed with no significant difference amongst the (b) (4) different LVs as measured by relative mean fluorescence intensity (MFI). These levels increased to ~70 - 80% of WT levels after transduction for all the hCD18-LVs demonstrating the ability of hCD18 to bind to the endogenous mCD11a subunit, forming a chimeric β_2 integrin.

Lin- BM cells transduced with Chim-CD18-WPRE LV transplantation into CD18^{HYP} Mice: At 4 months post-transplantation, percentages of hCD18⁺ harvested from Lin- BM cells transduced with Chim-CD18-WPRE LV ranged from 11 – 35% with similar results in the PB. Similar trends were observed in secondary recipients showing that the hCD18-LVs can transduce long-term HSCs (LT-HSCs). The Chim-CD18-WPRE LV showed the highest level of surface expression of hCD18 in myeloid cells when compared to the other LVs. The VCN/cell ranged from 0.4 – 0.9 in the PB leukocytes (PBLs) from primary and secondary transplant recipients, and the differences in hCD18 expression observed were not attributable to major differences in the number of integrated copies.

Neutrophil Extravasation: For the AP model, hCD18-LV transduced cells mice showed a statistically significant increase in the TNF- α -induced extravasation ratio that was observed in all hCD18-LV treated groups when compared to the (b) (4) control group in CD18^{HYP} mice. For the LPS-induced asthma model, while the donor extravasation ratio of CD18^{HYP} neutrophils was highly reduced in comparison with CD18^{WT} mice, a statistically significant increase in neutrophil extravasation ratio was observed in CD18^{HYP} mice transplanted with Chim-CD18-WPRE LV and (b) (4) Lin- BMCs.

Toxicology: Monthly observation for both primary and secondary recipients from the different studies in ex vivo hCD18 LV mediated gene therapy treated CD18^{HYP} mice showed no gross, physical, behavioral, or morphologic abnormalities. Different experiments showed no premature mortality or unexpected deaths over the course of the study period in either primary transplant recipients (i.e., 4-months) or in secondary transplant recipients (i.e., 9-months). This study duration was selected to assess any possible adverse/leukemogenic events, as these are murine cells, and the secondary transplant adds additional stress to the HSCs to enhance detection of any possible adverse events. Multilineage distribution in the PB at 4 months post-transplant of secondary recipients and age-matched UNT CD18^{WT} and CD18^{HYP} mice showed a similar profile as compared to WT reconstituted mice and all study animals presented with normal levels of WBCs.

Conclusions: Although the different hCD18-LVs evaluated demonstrated the ability to restore expression of CD18, the Chim-hCD18-WPRE LV conferred CD18 expression levels closer to physiologic levels and demonstrated preferential expression in mature myeloid lineages. Selection of the Chim-hCD18-WPRE LV could potentially minimize unintended off-target effects from ectopic expression driven by more ubiquitous promoter activity in the other LV

constructs. The Chim-hCD18-WPRE LV construct used for these experiments is the same that was used in clinical study NCT03812263.

Reviewer Comment:

- In humans, the CD34 selection marker is commonly used for positively identifying human HSCs. Most human CD34+ HSCs are also negative for lineage marker expression. In mice, a unique HSC selection marker does not exist. Lacking a positive cell marker such as CD34, mouse HSCs can be enriched based on the absence of lineage marker expression [i.e., Lin-: negative for CD3, B220, Gr1, macrophage-1 antigen (Mac-1), TER119]. Mouse Lin- cells were the most comparable population to the human CD34+ HSCs and were used to conduct the transplant experiments. Comparability of both selection procedures has been demonstrated as both populations comprise very primitive HSCs as well as more committed progenitors and are able to provide full hematopoietic reconstitution of recipients after myeloablative conditioning (Sergei Doulatov 2012).
- LAD-I is known to affect males and females in equal numbers. Per the applicant, female recipient mice were preferred for engraftment as survival is higher after irradiation and transplantation as well due to the behavioral characteristics of male mice; however, some male mice were also included (Faizy Notta, 2010). The applicant did not indicate the exact number of female or male mice used in this study.
- The inflammation induction model permits experimental assessment of neutrophil extravasation to demonstrate functional correction of LAD-I phenotypic features observed in CD18^{HYP} mice.

Study #4

Report Number		NSR-4
Date Report Signed		03-May-2023
Title		Assessment of in vivo engraftment of human hematopoietic progenitors transduced with Chim-CD18-WPRE lentiviral vector in NSG mice
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		<ol style="list-style-type: none"> 1. To transduce purified CD34+ HSCs derived from HD CB with the Chim-CD18-WPRE LV to demonstrate the ability of the vector to transduce HSPCs from HDs and test whether transduced cells can engraft and proliferate in NSG mice. 2. To demonstrate if any detrimental impact derived from transduction and potential overexpression of the human CD18 transgene.
Study Animals	Strain/Breed	NOD.Cg-(b) (4) ^{scid} IL2rg ^{(b) (4)} /SzJ (NSG mice)
	Species	<i>Mus musculus</i>
	Age	8 - 12 weeks
	Body Weight	~20 g Note: The sponsor did not provide initial body weight data for this study. The average body weight for this species was used instead.

	#/sex/group	Please see the study group and dose levels tables below for specific animal numbers per group that varied in terms of VCN, LVs (b) (4); Chim-CD18-WPRE LV), and the number of primary and secondary recipients. The exact number of males and females was not specified by the applicant.
	Total #	38
Test Article(s)		Chim-CD18-WPRE LV HD CD34+ HSCs
Control Article(s)		1. (b) (4) 2. UNT HD CD34+ HSCs (negative control)
Route of Administration		IV administration
Description of the Animal Model and Dose Levels		NSG mice were irradiated (1.5 Gy) 24 hours prior to transplantation and transduced or UNT CD34+ HSCs were transplanted ($\sim 4 \times 10^5$ cells/mouse). Human CD45+ cells were also isolated from the BM of each mouse to perform secondary transplantation in irradiated NSG mice ($\sim 4 - 8 \times 10^6$ cells/mouse).
Dosing Regimen		Single administration
Randomization		Yes
Description of Masking		No
Scheduled Sacrifice Time Points		Primary transplant NSG mice were euthanized 3 months post-transplantation. Secondary transplant NSG mice were euthanized 3 months post-transplantation.

Study Groups and Dose Levels:

Table 2. Human CD34+ HSCs transduced with Chim-CD18-WPRE LV for Hematopoietic Transplantation in NSG Mice

Condition	LV	No. of primary recipients	No. of secondary recipients
UNT	-	9	3
(b) (4)	(b) (4)	5	3
LVCN (low VCN)	Chim-CD18-WPRE LV	4	4
HVCN (high VCN)	Chim-CD18-WPRE LV	6	4

The LV.GFP group was transplanted with (b) (4) CD34+ HSCs at 4.35 VCN/cell. The low VCN (LVCN) group was transplanted with Chim-CD18-WPRE LV CD34+ HSCs at 0.44 VCN/cell. The high VCN (HVCN) group was transplanted with Chim-CD18-WPRE LV CD34+ HSCs at 2.79 VCN/cell.

Key Evaluations and Assessments⁹:

- Once a month, BM was collected to assess the levels of engraftment and multilineage distribution by flow cytometry.
- NSG mice, both primary and secondary, were sacrificed at 3 months post-transplant and the BM cells were evaluated for hematopoietic reconstitution and VCN.
- Note:** The sponsor did not include a description of the health status and/or clinical signs of the NSG mice.

Key Results:

Multilineage hematopoietic reconstitution: Similar repopulation ability and clonogenic potential was observed for the CD18 transduced groups as compared to the UNT and (b) (4) group, with an average of 40 – 60% of human CD45+ cells of the total engrafted population in primary recipients and lower but comparable levels observed in the secondary transplant, with no

significant differences observed after statistical analyses. The hCD18% expression in different hematopoietic cell lineages showed a similar distribution of CD19+ (lymphoid), CD33+ (myeloid), and CD34+ populations (progenitor cells) for the Chim-CD18-WPRE LV transduced HD CD34+ HSCs administered to NSG mice as compared to the (b) (4) and UNT controls. The expression of CD18 in different lineages was not affected by the level of transduction with Chim-CD18-WPRE LV (i.e., LVCN or HVCN) and the expression levels were sustained. The extracted gDNA from 3-month mouse PB and BM samples were also analyzed in study #9 (study report no. NSR-9) for ISA.

Conclusions: This study demonstrated that HD CB CD34+ HSCs transduced with the Chim-CD18-WPRE LV were capable of engraftment in NSG mice. Increased VCN from 0.44 VCN/cell (i.e., LVCN) to 2.79 VCN/cell (i.e., HVCN) of Chim-CD18-WPRE LV did not lead to increased levels of hCD18 on the cell membrane of transduced cells or their progeny, did not greatly impact their clonogenic potential, or in vivo hematopoietic engraftment of the repopulating cells.

Study #5

Report Number	NSR-5
Date Report Signed	03-May-2023
Title	Assessment of the use of transduction enhancers (b) (4) to improve Chim-CD18-WPRE LV transduction efficiency of CD34+ cells and maintain the reconstitution potential of the transduced cells
GLP Status	No
Testing Facility	(b) (4)
Objective(s)	<ol style="list-style-type: none"> 1. To test the combination of TEs (b) (4) to improve Chim-CD18-WPRE LV transduction efficiency of CD34+ cells derived from CB or mPB in vitro and to determine the suitability of TE incorporation in the manufacturing protocol to increase the VCN in gene-modified autologous CD34+ cells for LAD-I gene therapy. 2. To test the in vivo reconstitution potential of the CD34+ cells transduced with Chim-CD18-WPRE LV in the presence of TEs.
Strain/Breed	NOD.Cg-(b) (4) ^{scid} IL2rg ^{(b) (4)} /SzJ (NSG mice)
Species	<i>Mus musculus</i>
Age	8 - 12 weeks
Body Weight	~20 g Note: The sponsor did not provide initial body weight data for this study. The average body weight for this species was used instead.
#/sex/group	2 / Mock; 9 / Chim-CD18-WPRE LV w/o TEs; 8 / Chim-CD18-WPRE LV w TEs; 18 / Chim-CD18-WPRE LV w TEs from full scale transductions (Run I, II and III) Note: The exact number of males and females was not specified by the applicant.
Total #	37
Test Article(s)	CD34+ HSCs transduced with Chim-CD18-WPRE LV with TEs (i.e., (b) (4))
Control Article(s)	<ol style="list-style-type: none"> 1. CD34+ HSCs transduced with Chim-CD18-WPRE LV without TEs 2. UNT CD34+ HSCs

Route of Administration	IV administration
Description of the Animal Model and Dose Levels	CD34+ HSCs purified from human CB and from HD mPB were transduced at a MOI of 10 – 200 IU/cell with either transduction (b) (4) alone or in combination overnight (O.N.). In some experiments, transduced cells were cryopreserved and transplanted after thawing the final product. NSG mice were irradiated (1.5 Gy) 24 hours before and were subsequently transplanted with $1.3 - 2.9 \times 10^5$ cells/animal.
Dosing Regimen	Single administration
Randomization	Yes
Description of Masking	No
Scheduled Sacrifice Time Points	Mice were euthanized 3 months following CD34+ HSC transplantation

Study Groups and Dose Levels:

Table 3. Human CD34+ HSC transduced with Chim-CD18-WPRE (with or without TEs) for Hematopoietic Transplantation in NSG mice

Condition	Number of transplanted mice
Mock	2
Chim-CD18-WPRE LV w/o TEs	9
Chim-CD18-WPRE LV w TEs	8
Chim-CD18-WPRE LV w TEs from full scale transductions (Run I, II and III)	Run I (CB) (n = 6) Run II (mPB) (n = 7) Run III (CB) (n = 5)

Key Evaluations and Assessments¹⁰:

- Once a month, BM aspirations were conducted to assess the levels of BM engraftment and lineage distribution (i.e., CD45, CD33, CD34, CD19) as well as CD18 expression in the different hematopoietic populations.
- Transplanted mice were sacrificed 3 months post-transplant and total BMCs were similarly assessed for BM engraftment and lineage distribution as well as for VCN.
- Body weight was tracked for in-life assessment of recipient NSG mice. Macroscopic inspection was conducted following euthanasia.

Key Results:

Ex vivo engraftment in NSG mice: The TEs [i.e., (b) (4)] showed minimal impact on engraftment potential of gene-modified cells in the hematopoietic organs, as determined by comparable percentage of CD45+ cells in the BM of all groups analyzed. Similar proportions of CD34+ and CD33+ cells were noted in the BM of NSG recipients transplanted with the mock and Chim-CD18-WPRE LV samples. While a slightly higher proportion of CD19 cells was observed in mice transplanted with transduced cells, individual values obtained (37% - 65%) were in the upper range but were not higher than some of the values obtained in the group without TEs (range 19 – 70%) and was considered within the normal reconstitution variability amongst animals. No toxicity was observed for transplanted animals with no significant changes in body weight throughout the time of analysis and no macroscopic abnormalities or unexpected deaths. The TEs increased the in vivo VCN in NSG mice, achieving VCN > 1 when cells were transduced in the presence of TEs. To confirm the function of the HSPCs that had been transduced in good manufacturing practice (GMP) full scale runs, transduced CD34+ HSCs that

were cryopreserved for three months were thawed and transplanted into NSG mice. Mice were followed to monitor the engraftment of the gene-corrected cells. Flow cytometry analyses conducted in BM from recipient mice at the end of the follow-up period (3 months post-transplant) showed a mean value of $66.4 \pm 5.0\%$ and $55.3 \pm 14.6\%$ (Runs I and III, respectively) and $29.0 \pm 7.8\%$ (Run II) hCD45+ cells in mice transplanted with CB and mPB transduced cells, respectively. Quantitative polymerase chain reaction (PCR) analysis showed a mean VCN value of 1.1 ± 0.2 , 0.4 ± 0.1 , and 2.4 ± 0.4 copies per hCD45+ cells in Run I, Run II, and Run III, respectively. The presence of CD34+, CD19, and CD33+ human cells showed that the differentiation capacity of transduced cells was also maintained after cryopreservation.

Conclusions: Incorporation of TEs [i.e., (b) (4)] into the transduction protocol in vitro caused significant transduction level improvement in primitive HSPCs with long-term repopulating potential in vivo. The use of the TEs increased transduction efficiency without adversely impacting engraftment and multi-lineage differentiation capacity with stable gene marking of primitive stem cells and preserved repopulation potential and polyclonal integration patterns.

Reviewer Comment:

- Based on the supporting nonclinical data, the inclusion of TEs showed improved transduction efficiency. (b) (4), and protamine sulfate were used during the optimized clinical manufacturing (NCT03812263) as well as for the commercial manufacturing process.
- To determine the clonal composition of human engrafted cells, aliquots of transduced CD34+ HSCs (prior to transplantation) and BM cells obtained from representative recipients (from Run II, n = 5; from run III, n = 5) at the end of the follow-up period were used to perform ISA in study report no. 9 (NSR-9). Overall, the analyses of all samples obtained from in vitro transduced human CD34+ HSCs and CD34+ HSCs collected from transplanted NSG mice showed a polyclonal integration profile for Chim-CD18-WPRE LV. All transduced CD34+ HSCs samples pre- and post-transplantation showed polyclonality with strong clonal outgrowth in samples from transplanted mice (Mesa-Nunez, 2022).

Study #6

Report Number	NSR-6
Date Report Signed	03-May-2023
Title	Assessment of the use of transduction enhancers (b) (4) to improve Chim-CD18-WPRE LV transduction efficiency and in vivo phenotypic correction in LAD-I mouse models.
GLP Status	No
Testing Facility	(b) (4)
Objective(s)	To test the combination of TEs, (b) (4), to improve Chim-CD18-WPRE LV transduction efficiency while preserving HSC characteristics and to demonstrate the ability of the transduced cells to correct the neutrophil extravasation defect in the LAD-I mouse models in vivo.

Strain/Breed	1. B6.129S7-Itgb2 ^{tm2Bay/J} (CD18 ^{KO}) 2. B6.129S7-Itgb2 ^{tm1Bay/J} (CD18 ^{HYP}) 3. C57BL/6J ^{(b) (4)}
Species	<i>Mus musculus</i>
Age	8 - 12 weeks
Body Weight	~20 g Note: The sponsor did not provide initial body weight data for this study. The average body weight for this species was used instead.
#/sex/group	See Tables 4 and 5 below Note: The exact number of males and females was not specified by the applicant.
Total #	47
Test Article(s)	Murine HSCs transduced with Chim-CD18-WPRE LV and TEs (i.e., ^{(b) (4)})
Control Article(s)	1. Mock-transduced cells 2. UNT cells
Route of Administration	IV administration
Description of the Animal Model and Dose Levels	LSK cells (i.e., Lin-, Sca-1+, c-kit+) were transduced and IV administered 7,600 – 15,000 cells/mouse (assuming an average weight 20 g/mouse). This cell dose corresponds to 3.8 – 7.5 x 10 ⁵ cells/kg. Prior to transplantation, mice received a conditioning regimen to facilitate engraftment of donor cells. For CD18 ^{HYP} mice, lethal irradiation consisted of two consecutive doses of 4.5 Gy given the day before and the same day of transplantation. Sub-lethal irradiation was applied to CD18 ^{KO} recipients because of their high mortality rate, which entailed a single 7 Gy dose administered the day before the transplantation. The neutrophil extravasation ratio was determined using a localized LPS-induced pad inflammation model as compared to a PBS control pad. Control groups included untreated CD18 ^{WT} and CD18 ^{KO} mice. Inflammation in the LPS-treated pad was confirmed by measurement with a digital caliper and normalized to the PBS-treated pad. Each pad was processed, and the cellular suspension were stained to study the % of the Gr-1+CD11b+ and Gr-1+CD11b- populations as well as CD18 expression in the total hematopoietic population. The neutrophil extravasation ratio was also investigated using the LPS asthma model as previously described.
Dosing Regimen	Single administration
Randomization	Yes
Description of Masking	No
Scheduled Sacrifice Time Points	Mice were euthanized at 4 months.

Study Groups and Dose Levels:

Table 4. LAD-I-like Animal Models used for Ex vivo Gene Therapy Experiments

Strain	Gender	Strain	Gender	Irradiation	Transplanted cells/mouse
CD18 ^{HYP}	Male/Female	CD18 ^{HYP}	Male/Female	Lethal (4.5+4.5 Gy)	7,600 – 11,000
CD18 ^{KO}	Male	CD18 ^{KO}	Female	Sub-lethal (7 Gy)	15,000
C57BL/6J	Male	CD18 ^{KO}	Female	Sub-lethal (7 Gy)	15,000

Gy: Grays

Table 5. Ex Vivo Gene Therapy Experiments in CD18^{HYP} Mice Infused with CD18^{HYP} LSK Cells Transduced in the Presence of TEs

MOI	LVV production	Donor cells	Vector	TEs	Condition	CD18 ^{HYP}	Live recipients at the end of follow-up
0	-	-	-	-	UNT	4	4
20	(b) (4)	CD18 ^{HYP}	LV:Chim.hCD18	-	w/o TEs	9	9
	(b) (4)	CD18 ^{HYP}	LV:Chim.hCD18	(b) (4)	w/ TEs	13	13

Lethally irradiated CD18^{HYP} recipients were transplanted with Chim-CD18-WPRE LV-transduced CD18^{HYP} LSK cells in the presence with or absence of TEs [i.e., (b) (4)]. Abbreviations: HYP, hypomorphic; LV, lentiviral vector; MOI, multiplicity of infection; UNT, untransduced.

Table 6. Ex vivo Gene Therapy Experiments in CD18^{KO} Mice Infused with CD18^{KO} LSK Cells Transduced in the Presence of TEs

Condition	MOI	LV Production	Transplanted cells (males)	Donor cells	Transduction Enhancers	Number of CD18 ^{KO} female recipients	Live recipients at the end of follow-up
CD18 ^{WT} UNT	0	-	15,000	CD18 ^{WT}	-	5	2
CD18 ^{KO} UNT	0	-	15,000	CD18 ^{KO}	-	7	2
CD18 ^{KO}	20	(b) (4)	15,000	CD18 ^{KO}	(b) (4)	9	5

Control groups included CD18^{WT} mice transplanted with UNT CD18^{WT} cells and CD18^{KO} mice transplanted with UNT CD18^{KO} donor cells. The test group include CD18^{KO} mice transplanted with CD18^{KO} cells transduced with MOI (20 TU/cell) of the Chim-CD18-WPRE LV in the presence of TEs. Concentrations of TEs used in this experiment were the following: (b) (4). Abbreviations: LV, lentiviral vector; MOI, multiplicity of infection.

Key Evaluations and Assessments¹¹:

- Recipients were followed for 3 – 4 months and bled monthly for integrin expression and leukocyte subpopulation analyses of reconstituted T cell (CD3e+), B cell (B220+) and granulocytes (Gr-1+/Mac-1+) as measured by flow cytometry and VCN analyses.
- At the end of the experimental procedure, transplanted mice were evaluated for phenotypic gene correction using the LPS-induced asthma inflammation models as previously described in study report no. 3. The LPS-induced pad inflammation model was also conducted to evaluate LV-mediated gene correction of neutrophil extravasation.

Key Results:

Ex vivo Gene Therapy Experiments in CD18^{HYP} Mice: Differences in hCD18 expression were present at 1-month post-transplantation, with an average of $15.93 \pm 3.58\%$ hCD18+ cells

observed in the group transplanted with cells transduced in the absence of TEs versus $22.83 \pm 4.21\%$ hCD18⁺ cells found in the group with TEs. At 3.5 months post-transplantation, the hCD18⁺ cell % values were $9.22 \pm 4.75\%$ in the group without TEs and $19.87 \pm 7.78\%$ in the group with TEs. At 3.5 months post-transplant, the PB samples of these mice similarly correlated with the hCD18⁺ cell% value with 0.18 ± 0.09 VCN/cell without TEs and 0.33 ± 0.17 VCN/cell with TEs. The stability of transgene expression and VCN/cell in the group transplanted with cells transduced in the presence of TEs suggested that these compounds enhance transduction efficiency of primitive HSPCs responsible for the engraftment in these animals. PB samples were also evaluated for the expression of hCD18 and for different mCD11 subunits and expression of normal levels of CD18 correlated with restored CD11 expression. The hCD18:mCD11a heterodimer [i.e., Lymphocyte function associated antigen-1 (LFA-1)] showed greater expression levels in the recipients of cells transduced with TEs ($17.59 \pm 4.32\%$) as compared to recipients of cells without TEs ($9.19 \pm 3.65\%$) and the values reached significance at 3.5 months post-transplantation. Transgene expression in myeloid cells (Ly6G⁺/Mac-1⁺ population) was almost 2 times greater than levels reached in lymphoid lineages (CD3e⁺ and B220⁺ populations), both for recipients of cells transduced in the absence of TEs (1.94-fold increase) and in the presence of TEs (2.41-fold increase). This result correlated with the activity of the 'Chim' promoter, which is preferentially active in myeloid cells. For the Ly6G⁺/Mac-1⁺ population, increased hCD18⁺ expression MFI in the presence of TEs versus in the absence of TEs was greater than the difference observed in other cell lineages. Although the values obtained were lower than those found in the PB, the VCN/cell in BM also reflected a significantly greater transduction in the group transplanted with cells transduced with TEs as compared to the group receiving the cells transduced without TEs.

Neutrophil Extravasation in LPS-induced Asthma Model in CD18^{HYP} Mice: A tendency for a greater neutrophil migration to the lung in gene therapy-treated mice was observed as compared to control CD18^{HYP} mice or CD18^{HYP} mice transplanted with UNT CD18^{HYP} LSK cells, demonstrating that neutrophils in all gene therapy treated groups were able to restore their ability to extravasate from the PB to inflamed tissues. More extensive analyses showed that 6 out of 9 mice (66.7%) showed evidence of neutrophil migration in the gene therapy-treated group in the absence of TEs, whereas for the gene therapy-treated group transduced in the presence of TEs, all treated mice (n = 8, 100%) displayed neutrophil migration to the lung.

Neutrophil Extravasation in an LPS-induced Pad Model in CD18^{KO} Mice: While almost no expression of the α and β subunits (hCD18) of β_2 integrins was observed in PB cells from untransduced recipient CD18^{KO} mice, $24.1 \pm 3.1\%$ of PB cells from mice that received transduced cells were hCD18⁺. In these mice, a mean VCN of 0.4 ± 0.1 copies/cell was observed, indicating that a greater proportion of transduced cells expressed hCD18. Greater expression of hCD18 was observed in the myeloid PB cells compared with T lymphocytes, resembling the physiological expression of this β_2 integrin (Diego Leon-Rico, 2016). The expression of hCD18 also correlated with the expression of mCD11a in gene therapy treated CD18^{KO} mice. Only a very low proportion of PB cells were positive for CD11c expression (i.e., dendritic cells). While in no instance were Gr1⁺CD11b⁺ neutrophils observed in the LPS-challenged pads from CD18^{KO} mice, in gene therapy-treated CD18^{KO} mice, the proportion of leukocytes (CD45.2⁺ cells) consisting of infiltrating neutrophils reached 40% of values as compared to PBS-treated pads from WT mice (p < 0.05). In LPS-treated pads, the proportion of

infiltrating neutrophils increased in both groups, and no difference between the WT and the gene therapy-treated CD18^{KO} mice were observed. These results are consistent with those observed in gene therapy-treated CD18^{HYP} mice, which exhibited greater migration of neutrophils to the lung as compared to the control CD18^{HYP} mice. Overall, these results suggest that gene therapy restored β_2 integrin expression and β_2 integrin-dependent migration of CD18^{KO} neutrophils toward inflamed pads and the use of TEs can improve gene correction in CD18^{KO} mice.

Reviewer Comment:

- Utilization of TEs [i.e., (b) (4)] during the Chim-hCD18-WPRE LV transduction of murine CD18^{HYP} HSCs, improved the genetic correction of these cells and enhanced neutrophil extravasation in an LPS-induced lung inflammation model in recipient CD18^{HYP} mice. Similar results were observed in the more severe CD18^{KO} mouse model with stable engraftment of transduced HSCs and enhanced neutrophil extravasation using an LPS-induced pad inflammation model. These results confirm that the use of TEs can improve genetic correction in the CD18^{HYP} and CD18^{KO} mouse models.

SAFETY PHARMACOLOGY STUDIES

Safety pharmacology studies were not conducted for this product.

Pharmacokinetic Studies (Cell Distribution)

Assessment of the cell distribution profile of Chim-CD18-WPRE LV transduced murine HSPCs was incorporated into Study #7 (study report no. NSR-7) reviewed in the Toxicology Studies.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology and BD study was conducted to evaluate the safety of the Chim-CD18-WPRE LV vector-transduced murine HSPCs.

Toxicology Study:

Study #7

Study Number	Study Title / Publication Citation	Report Number
7	Assessment of biodistribution and potential toxicity and presence of replication competent lentiviral vector from Chim-CD18-WPRE lentiviral vector transduced hematopoietic stem and progenitor cells in a murine hematopoietic transplant model	NSR-7

Developmental and Reproductive Toxicology Studies¹²:

Studies were not conducted to evaluate this developmental and reproductive toxicity based on the product type and due to the lack of concerning findings in reproductive tissues in Study #7 (study report no. NSR-7). The applicant showed BD to reproductive tissues was near or below the background threshold, which is indicative of a low risk of LV-mediated germline

transmission to reproductive tissues as supported by previous publications (Ilaria Visigalli, 2016).

Genotoxicity Studies:

Study Number	Study Title / Publication Citation	Report Number
8	Analysis of Integration Profile in Mouse Transduced Cells	NSR-8
9	Analysis of Integration Profile in Human Transduced Cells	NSR-9

Carcinogenicity/Tumorigenicity Studies:

Traditional carcinogenicity studies were not conducted since they are not suitable for assessment of this drug product type. The nonclinical data provided by sponsor showed no evidence of tumorigenicity in the in vivo studies performed or preferential integration near proto-oncogenes in the integration site analysis (ISA).

Toxicology Studies

Study #7

Report Number	NSR-7
Date Report Signed	03-May-2023
Title	Assessment of biodistribution and potential toxicity and presence of replication competent lentiviral vector from Chim-CD18-WPRE lentiviral vector transduced hematopoietic stem and progenitor cells in a murine hematopoietic transplant model
GLP Status	No
Testing Facility	(b) (4)
Objective(s)	To characterize the BD and potential toxicity of HSCs transduced ex vivo with a pre-GMP Chim-CD18-WPRE LV in a murine HSPC transplantation model. An additional objective of this study was to confirm the absence of replication competent lentivirus (RCL) in animals transplanted with Chim-CD18-WPRE LV-transduced murine HSPCs.
Species	<i>Mus musculus</i>
Age	8 – 12 weeks
Body Weight	~20 g Note: The sponsor did not provide initial body weight data for this study. The average body weight for this species was used instead.
#/sex/group	See Table 8 below
Total #	52
Test Article(s)	Chim-CD18-WPRE LV transduced Lin- BM cells
Control Article(s)	UNT Lin- cells
Route of Administration	IV administration
Description of the Mouse Model and Dose Level	Mice were transplanted with 1.5×10^7 Lin- transduced cells isolated from (b) (4) donor mice. Male and female control groups received UNT cells.
Dosing Regimen	Single administration
Randomization	Yes
Description of Masking	No
Scheduled Sacrifice Time Points	Mice were sacrificed 1 month after Chim-CD18-WPRE LV transduced Lin- cell transplantation.

Strain/Breed:

Table 7. List of Recipient Mouse Strains

Strain	Type	Background	CD45	Experiments
(b) (4)	Hybrid mice	(b) (4) male	(b) (4)	Recipients for biodistribution studies. Males and females.
(b) (4)	Hybrid mice	(b) (4) male	(b) (4)	Lin- BM donors for biodistribution experiments. Males and females.

Study Groups and Dose Levels:

Table 8. Cohorts of Transplanted Mice in the BD Assay

Donor Lin-cells	(b) (4) Donor	Male (b) (4) recipients	Female (b) (4) recipients
Cohort I	Chim-CD18-WPRE LV	11	11
	UNT control	6	6
Cohort II	Chim-CD18-WPRE LV	8	6
	UNT control	2	2

Key Evaluations and Assessments¹³:

- Mice were followed for 1 month. During this period, mice were monitored daily, and weights were annotated weekly.
- At 1-month post-transplant, PB and BM were collected for measurement of hCD18 expression and flow cytometry analyses of the different hematopoietic lineages, VCN and hematology counts. Likewise, hematopoietic tissues (i.e., bone marrow, spleen, thymus, and lymph nodes) and non-hematopoietic organs (i.e., heart, liver, kidney, gonads, intestine, and brain) were collected and processed for flow cytometry and VCN.
- PB serum was collected for biochemical profiling and human immunodeficiency virus (HIV) p24 measurement via (b) (4) for RCL detection.
- The spleen, lymph nodes, thymus, and gonads were collected for histopathology. Perfused organs including the liver, kidney, intestine, lungs, heart, muscle, pancreas, and brain were also collected for VCN and histopathology.

Key Results:

In-life assessment: The test article treated mice showed no physical, behavioral, or morphological abnormalities. No premature mortality or unexpected deaths were observed over the course of the study period in any recipient mice for a 1-month study duration. Animals transplanted with transduced cells recovered weight normally after transplant similar to those

transplanted with UNT cells. Transplanted mice that received Lin- BM cells transduced with Chim-CD18-WPRE LV showed favorable engraftment 1-month post-transplantation and normal levels of WBCs comparable to animals transplanted with UNT cells. In study cohort I, normal multilineage distribution in the PB was also observed as compared to animals reconstituted with UNT cells.

VCN and Transgene expression: WBC counts in both the PB and BM showed no significant differences amongst transduced cells and control groups in both sexes. Both males and females showed ~70 – 95% engrafted cells, as evaluated by the presence of CD45.1 positive donor cells, in the PB and BM, along with other hematopoietic organs analyzed (i.e., spleen, thymus, and lymph nodes). Analyses of BM and PB cells showed an average of 30% of hCD18+ cells in the BM of engrafted animals and 25% of hCD18+ cells in the PB from all transplanted animals in study Cohort I. Similar results were observed in study Cohort II with ~23% of hCD18+ cells in the BM and ~14% of hCD18+ cells in the PB. The average VCN/cell detected was 0.9 in the BM cells and 0.8 in the PB cells in Cohort I with an average of 0.5 VCN/cell detected in both the BM and PB in cohort II. These values were comparable to the VCN in transduced Lin- cells prior to transplantation (average 1.1 VCN/cell in Cohort I and average 0.5 VCN/cell in Cohort II).

Non-hematopoietic organs showed undetectable VCN and any values above zero were attributed to the presence of residual hematopoietic extravasated cells residing in different tissues, particularly in the lungs and intestines. To confirm the absence of proviral copies in the gonads, these organs were analyzed in more detail in Cohort II of transplanted mice. The residual hematopoietic cells present in the gonads was variable and always lower than 10%, correlated with a VCN at or near background levels, providing additional BD data to support the lack of germline transmission risk.

Clinical pathology: No significant alterations in the serum biochemical profiles were observed between the groups of transplanted animals.

Histopathology: Many of the liver sections had Grade 1 and occasional Grade 2 infiltration of mononuclear inflammatory cells. However, cellular infiltrates are a common background liver finding of laboratory mice and were not considered to be of pathological significance. All histological sections of the testis, including both the transduced and UNT specimens, had marked degeneration of the seminiferous tubules. The degeneration of the seminiferous tubules is a known adverse effect of mouse irradiation for pre-conditioning regimen prior to Lin- cell transplantation. There were no differences in the degree of degeneration between the mice receiving the untransduced cells and those receiving the transduced cells, suggesting that this effect was attributable to the irradiation protocol rather than the investigational product. The remaining tissues were within normal histological limits.

p24 (b) (4) for RCL characterization: To demonstrate the absence of shedding of the LV, serum was collected from the transplanted mice in study Cohort II and evaluated for the presence of vector derived from HIV p24 to address the risk of RCL. The p24 levels were under the detection threshold in all transplanted animals. Negative results for vector derived p24 determination suggested that no RCL LV was generated because of a recombination process and demonstrated the absence of persistent vector particles in the transplanted animals.

Conclusions: No adverse findings were observed following the infusion of the surrogate Chim-hCD18-WPRE LV-transduced Lin- HSPCs into irradiated recipient mice. The results obtained from this study support the use of the Chim-hCD18-WPRE LV for transduction of HSPCs. The transduction protocol and transduction cell dose included a high MOI of 100 IU/cell representative of the upper limit used in the commercial manufacturing (b) (4) IU/cell).

Reviewer Comments:

- Purified Lin- BM cells transduced with Chim-CD18-WPRE LV were used as surrogate cells for human CD34+ HSCs using a high cell number (1.5×10^7 cells/mouse) and VCN (0.7 – 0.9 VCN/cell) to evaluate safety by monitoring for physical, behavioral, biochemical, or morphological abnormalities in recipient mice.
- The study duration of 1 month after transplantation is considered an optimal timeframe to observe hematopoietic reconstitution and BD of the LV in the non-hematopoietic organs and potential RCL after a high vector load in transduced cells. However, the 1-month timeframe may not be sufficiently long enough to detect tumors or associated malignancies in recipient mice.
- The absence of proviral copies in the gonads is also corroborated by previous nonclinical evaluations and clinical studies of gene therapies with LVs in HSCs in which the absence of proviral copies in germline tissues and lack of germline transmission of the provirus has been demonstrated (Ilaria Visigalli, 2016).

Study #8

Objective: To conduct ISA using non-restrictive linear amplitude-mediated polymerase chain reaction [(nr)LAM-PCR] and subsequent deep sequencing to determine the integration profile, characteristics, and potential clonal dominance.

Study Design: Lin- cells of LAD-I deficient mice (i.e., CD18^{HYP}) were transduced with the Chim-CD18-WPRE LV (b) (4) and gDNA was extracted from the transduced cells, PB, and BM samples. ISA was subsequently conducted for different post-transplantation timepoints (i.e., 1, 4, or 9 months) from primary and secondary recipients. Samples were prepared and selected from animals with sufficient Chim-CD18-WPRE LV transduction levels (0.05 – 0.2 VCN) suitable for ISA.

Results: Analysis of samples obtained from the BM of all secondary recipients showed an oligoclonal to polyclonal vector integration profile based on gel images, unique integration site number, and integration site sequence retrieval frequencies of the 10 strongest clones up to 9 months post-transplantation. A total of 304 unique exactly mappable IS could be detected from the BM sample of secondary recipients at 9 months post-transplantation. Semi-quantitative PCR revealed a VCN in the range of 0.05 – 0.2 for all samples except Chim6, where the VCN was not detectable (< 0.01). No enrichment of IS for a particular genomic region was found that would suggest selective vector mediated clonal outgrowth. There were no integration events detected in or nearby genes previously involved in serious adverse events (SAEs) in gene therapy such as MDS1, EVI-1, LMO2 (Adrian Schwarzer, 2021). Common integration sites (CIS) of order 4 (4 individual IS found within one sub-genomic region) were most exclusively found within one

cohort. Only two CIS of order 4 (genes FBXO8 and Gm156) were each comprised of IS from two different samples derived from the same Lin- cells.

Conclusions: The ISA of primary and secondary transplant samples from LAD-I deficient mice showed an oligoclonal to polyclonal vector integration profile for the Chim-hCD19-WPRE LV up to 4-month post-transplantation. A total of 2,398 unique exactly mappable IS were retrieved over the entire follow-up time (up to 4 months) after transplantation. There were no preferred IS in/nearby genes previously associated with insertional mutagenesis in gene therapy trials such as MECOM, LMO2, and CCND2. Genes affected by the top 10 CIS were also not associated with any cancer or clonal expansion/dominance event.

Reviewer Comment:

- Across multiples studies, no unexpected deaths or insertional mutagenesis events were observed over the course of the study period in either primary transplant recipients (4-months post-transplantation) or in secondary transplant recipients (5-months after secondary transplantation). Integration profiles indicated no evidence of clonal expansion due to transactivation of oncogenes over time in primary and secondary transplant recipients, with no appearance of leukemia in any of the recipient mice.

Study #9

Objective: To conduct ISA via shearing extension primer tag selection ligation-mediated-polymerase chain reaction (S-EPTS/LM-PCR) to identify IS within or in the vicinity of proto-oncogenes that have been implicated in tumor development in patients treated with gene therapy for this LV transduced human CD34+ HSC product.

Study Design: Human CD34+ HSCs derived from CB were transduced with the Chim-hCD18-WPRE LV and were transplanted into NSG mice. In vitro transduced cells were harvested after 14 days of expansion for analysis of CB CD34+ cells. Likewise, murine PB CD34+ HSCs were collected 3 months post-transplantation. A list of well-defined cancer associated genes were compiled from the Cancer Census database (<http://cancer.sanger.ac.uk/census>; last update was the 8th of May 2018, v85), which identified genes based on how mutations in these genes drive progression to cancer (i.e., 1) amplifications, 2) large deletions, 3) splice site mutations, 4) genes that have been identified as translocation partners with oncogenic genes). IS detected within a 100 kb window of a transcription start site (TSS) of pre-selected cancer-related and SAE genes were plotted by sample against the frequency of occurrence in the total amount of reads.

Results: All samples obtained from in vitro and in vivo studies using human CD34+ HSCs showed a polyclonal integration profile for the Chim-hCD18-WPRE LV according to unique integration site number and sequence retrieval frequencies of the ten strongest IS. A total of 27,961 (CIE02GW) and 45,444 (CIE03GW) unique exactly mappable IS were determined by high-resolution S-EPTS/LM-PCR in all samples. The highest number of IS were found in both pre-transplantation samples (26,245 in Run III LC and 43,987 Run II LC), whereas the number of IS was lower in samples from transplanted mice (93 to 466 in Run III and 108 to 376 in Run II). The relative contribution of IS were below 0.09% in both pre-transplantation samples. The

strongest relative contribution amongst samples from transplanted mice in Run II was found in sample Run III 3 for NUDT3 (13.592%); sample Run II 1 for YLPM1 (36.359%) of all IS sequence counts from this sample. A total of 2.91% IS from Run III and 2.47% IS from Run II were close to pre-defined, well characterized genes listed in the Cancer Gene Census database. The strongest occurrence was observed for gene RUNX1 (2.065% in sample Run III 5; nearest gene SETD4) and STAG2 (14.411% in sample Run II 5). A total of 39 IS in Run II samples and 13 IS in Run III samples were found within or close to proto-oncogenes CCND2, MECOM, LMO2, and MN1, which were involved in the development of SAEs (clonal dominance, leukemia, or MDS) in previous gene therapy trials. However, due to the polyclonal composition of all samples, the individual contributions were very low (0.001 to 0.127%).

Conclusions: Overall, S-EPTS/LM-PCR allowed high sequencing depth, which resulted in high counts of unique IS. All transduced CD34+ HSCs pre- and post-transplantation showed polyclonality with stronger clonal outgrowth in samples from transplanted mice. Although some IS were found within or close to proto-oncogenes CCND2, MECOM, LMO2, and MN1 associated with SAEs in gene therapy trials, there was no preferential integration observed, and their individual contribution was very low due to polyclonal composition of all samples.

Reviewer Comments:

- The cells used in this study were derived from HD and were not isolated from LAD-I patients and it is unclear whether there may be differences in the vector integration profile between HD and LAD-I patients.
- The longest evaluation time point in this study was 90 days. This may not be a sufficient length of time to detect integration events associated with clonal dominance, leukemia, or MDS as such events are sometimes detected at later follow-up time points during clinical trials.

APPLICANT'S PROPOSED LABEL

Section 13.1 ('Carcinogenesis, Mutagenesis, Impairment of Fertility') should be revised to only include information from the nonclinical studies necessary for the safe and effective use of the product.

Section 12.1 ('Mechanism of Action') The statements in this section should be revised to remove reference of preferential expression of CD18 in myeloid cells as it was based on P/T data using surrogate mouse BMCs in CD18^{HYP} mice and is not necessarily reflective of the intended clinical product.

CONCLUSION OF NONCLINICAL STUDIES¹⁴

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

KRESLADI, marnetegrage autoemcel, lentiviral vector, hematopoietic stem cells, human CD18, leukocyte adhesion deficiency I

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