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I concur with this review memo. Danielle Brooks 12/8/25

I concur with this review memo. A. Wensky. 12/8/2025

**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: 125846

STN #125846.000

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PRODUCT: WASKYRA (Etuvetidigene autotemcel) suspension for intravenous infusion

APPLICANT: Fondazione Telethon ETS/FTE
PROPOSED INDICATION: WASKYRA is an autologous hematopoietic stem cell-based gene therapy indicated for the treatment of pediatric patients aged 6 months and older and adults with Wiskott-Aldrich Syndrome (WAS) who have a mutation in the WAS gene for whom hematopoietic stem cell transplantation (HSCT) is appropriate and no suitable human leukocyte antigen (HLA)-matched related stem cell donor is available.

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EXECUTIVE SUMMARY:

WASKYRA™ (Etuvetidigene autotemcel) consists of autologous CD34+ hematopoietic stem and progenitor cells (HSPCs) transduced with a replication-deficient 3rd-generation self-

inactivating lentiviral vector (SIN-LVV) indicated for the treatment of Wiskott-Aldrich Syndrome (WAS). The WAS gene functions as a regulator of actin cytoskeleton reorganization, which is critical for multiple cellular functions in hematopoietic cells including adhesion, migration, phagocytosis, and immune synapse formation. Wiskott-Aldrich Syndrome Protein (WASP) deficiency contributes to a reduced number and impaired function of T lymphocytes, insufficient antibody or abnormal autoantibody production, defective natural killer (NK) cell function, reduced chemotaxis of phagocytes and dendritic cells (DCs), functional deficits in regulatory T cells, and abnormal platelet development with thrombocytopenia and reduced platelet size. Following intravenous (IV) infusion, WASKYRA™ is anticipated to engraft in the bone marrow (BM) and differentiate into various hematopoietic cell lineages thereby expressing sufficient levels of WASP to restore immune cell function and platelet count.

In vitro pharmacology studies showed that analogous WAS LVV transduced CD34+ HSPCs retained their multilineage differentiation potential while restoring T cell, B cell, and DC function. In vivo pharmacology studies conducted using surrogate lineage negative (Lin-) BM cells transduced with WAS LVV transplanted into WAS-deficient mice demonstrated increased B cell, platelet, and granulocyte cell counts and improved T cell, B cell and DC function. This also resulted in reduced autoantibodies and ameliorated gastrointestinal colitis.

In vivo pharmacokinetic studies were conducted using human HSPCs transduced with WAS LVV transplanted into neonatal Recombination Activating Gene 2 Interleukin-2 (IL-2) receptor gamma chain (Rag2^{-/-}IL2rg^{-/-}) mice permissive to hematopoietic engraftment and multilineage differentiation. The transplanted human HSPCs differentiated into both lymphoid and myeloid lineages in various hematopoietic organs. WAS LVV biodistribution was limited to the hematopoietic compartment with no evidence of bystander transduction in non-hematopoietic cells or germline transmission and no evidence of vector integration in murine cells.

In vivo long-term engraftment in primary or secondary WAS-deficient gene therapy (GT) bone marrow transplant (BMT) recipient mice showed no adverse findings in survival. Tumors were either of non-hematopoietic origin or lymphomas were of host origin and showed no evidence of LVV integration. Gene integration analysis showed diverse insertion patterns without clustering near oncogenes or tumor suppressor genes. This polyclonal distribution was observed in both patient-derived CD34+ cells and immunodeficient mouse models, indicating favorable safety characteristics.

No carcinogenicity, developmental and reproductive toxicology (DART) studies, and safety pharmacology studies were conducted for Etuvetidigene autotemcel. These studies are not warranted based on the drug product characteristics and safety profile.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

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

Formulation and Chemistry:

Etuvetidigene autotemcel is a cell suspension for IV infusion. Its active drug substance consists of mobilized autologous CD34+ HSPCs isolated from WAS patients transduced by a LVV encoding the WAS complementary deoxyribonucleic acid (cDNA) sequence for expression of WASP in hematopoietic cell lineages (Figure 1). Etuvetidigene autotemcel consists of a minimum recommended dose of 7×10^6 CD34+ cells/kg administered based on the patient's body weight at the time of infusion. Etuvetidigene autotemcel is cryopreserved in 5% v/v dimethyl sulfoxide (DMSO), 7% weight/volume (w/v) human serum albumin (HSA) in a 0.9% w/v saline solution.

(b) (4)

List of Abbreviations

ADME	Absorption, Distribution, Metabolism, Excretion
B2-SINE	B2 Short Interspersed Nuclear Element
bp	Base Pair
BLA	Biologics License Application
BM	Bone Marrow
BMDC	Bone Marrow Dendritic Cell
BMT	Bone Marrow Transplant
BL6	C57BL/6
CV	Cardiovascular
CNS	Central nervous system
CFU	Colony Forming Unit
CFU-C	Colony Forming Unit Cells
cDNA	Complementary DNA
CI UM	Control Item Designation: Unmanipulated
CI UT	Control Item Designation: Untransduced (Mock)
DC	Dendritic Cell
DNA	Deoxyribonucleic Acid
DART	Developmental and Reproductive Toxicology
DMSO	Dimethyl sulfoxide
dsDNA	Double-stranded DNA
EF1 α	Elongation factor 1-alpha
ELISA	Enzyme-Linked Immunosorbent Assay
F	Female

FACS	Fluorescence-Activated Cell Sorting
GT	Gene Therapy
GMP	Good Manufacturing Practice
GvHD	Graft-Versus-Host Disease
(b) (4)	
Gag-p24	HIV-1 Group Specific Antigen – generating p24 viral core structural protein
HD	Healthy Donor
HSPC	Hematopoietic Stem and Progenitor Cell
HSC	Hematopoietic Stem Cell
HSCT	Hematopoietic Stem Cell Transplantation
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus Type 1
HLA	Human Leukocyte Antigen
(b) (4)	
IgM	Immunoglobulin M
ISA	Insertion Site Analysis
IFN- γ	Interferon- γ
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-10	Interleukin-10
IV	Intravenous
IVIM	In vitro immortalization
kb	kilobase
LM/LAM-PCR	Ligation-mediated/linear amplification-mediated PCR
Lin-	Lineage Negative
LV	Lentivirus / Lentiviral
LVV	Lentiviral Vector
LTC-IC	Long-Term Culture-Initiating Cells
LTR	Long Terminal Repeat
M	Male
mPB	Mobilized Peripheral Blood
MOI	Multiplicity of Infection
NK	Natural Killer
NSG	Non-obese diabetic severe combined immunodeficiency IL-2 receptor common gamma chain
(b) (4)	
PB	Peripheral Blood
PK	Pharmacokinetic
PGK	Phosphoglycerate kinase 1
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RAD	Radiation Absorbed Dose
RT-qPCR	Real-Time Quantitative Polymerase Chain Reaction
Rag2 ^{-/-} IL2rg ^{-/-}	Recombination Activating Gene 2 Interleukin-2 receptor gamma chain

RCL	Replication Competent Lentivirus
RNA	Ribonucleic Acid
SIN-LVV	Self-inactivating lentiviral vector
SCID	Severe Combined Immunodeficiency
SFFV	Spleen Focus-Forming Virus
TCR	T Cell Receptor
TI	Test Item Designation: Transduced
TNF- α	Tumor Necrosis Factor- α
UCB	Umbilical Cord Blood
UM	Unmanipulated
UT	Untransduced (or Mock)
VCN	Vector Copy Number
VSV-G	Vesicular Stomatitis Virus Glycoprotein G
v/v	Volume/Volume
w/v	Weight/Volume
WT (or wt)	Wild Type
WAS	Wiskott-Aldrich Syndrome
WASP	Wiskott-Aldrich Syndrome Protein
WIP	Wiskott-Aldrich Syndrome Protein Interacting Protein
WKO	Wiskott-Aldrich Syndrome Protein Knockout
WPRE	Woodchuck Hepatitis Virus Post-Transcription Regulatory Element
(b) (4)	

Related File(s):

- **IND#18919:** Autologous CD34+ Cells Transduced with Lentiviral Vector Expressing Human Wiskott-Aldrich Syndrome Gene; Telethon003 (formerly OTL-103 and GSK2696275); Treatment of Wiskott-Aldrich Syndrome; Fondazione Telethon ETS

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INTRODUCTION

WAS is a rare X-linked primary immunodeficiency with an estimated incidence of 1:250,000 males, which is characterized by thrombocytopenia and associated bleeding, eczema, recurrent and severe infections, autoimmunity, and lymphoreticular malignancies (i.e., lymphomas, leukemias, and myelodysplasias) (H Ochs, 2006). WAS patients experience a severely reduced life expectancy of ~38% survival at 16 years and median survival of 14.5 years with the main causes of death being attributed to severe infections (44%), malignancies (26%), and hemorrhages (23%) (S Dupuis-Girod, 2003). The disease is caused by mutations in the WAS gene that encodes WASP, a protein essential for actin cytoskeletal reorganization exclusively expressed by hematopoietic cells and plays an important role in multiple cellular functions including adhesion, migration, phagocytosis, immune synapse formation, and receptor-mediated cellular activation (L Dupre, 2006). Defective WASP expression causes functional impairments in hematopoietic precursors, platelets, T cells, B cells, NK cells, DCs, macrophages, neutrophils, and mast cells.

Currently, there is no FDA-approved treatment for WAS. Allogeneic hematopoietic stem cell transplantation (HSCT) from a HLA identical donor or matched unrelated donors is the only known curative treatment for WAS. Patients lacking a related identical donor, or a matched unrelated donor may undergo BMT from a mismatched related donor (A Filipovich, 2001). However, complications from Epstein-Barr virus lymphoproliferative syndrome, graft rejection, chronic graft-versus-host disease (GvHD), autoimmunity, and infections can contribute to reduced survival in treated WAS patients (H Ozsahin, 2008).

After the WAS patient receives a reduced intensity conditioning regimen, Etuvetidigene autotemcel is infused, and transduced CD34+ HSPCs engraft in the BM subsequently differentiating into various hematopoietic cell lineages that express sufficient levels of WASP to restore immune cell function and platelet count.

NONCLINICAL STUDIES

Products evaluated in the nonclinical studies

The initial in vitro and in vivo studies were conducted using a previous (b) (4) version of the WAS LVV. This vector was a (b) (4) encoding WAS under (b) (4). This construct is identical to the intended clinical product (b) (4); abbreviated as (b) (4) except for a (b) (4).

(b) (4) of the clinical product (WASKYRA) to reduce the potential for oncogenicity as well as a (b) (4), which is not (b) (4) or (b) (4). The two vector constructs were determined to be sufficiently comparable to the clinical product to inform safety and activity in the pharmacology studies. The final intended clinical vector (Etuvetidigene autotemcel) was used in the definitive nonclinical studies including the in vivo toxicology and biodistribution studies. Other vectors expressing the WAS gene under control of ubiquitous promoters were also used during the initial development and testing of the LVV constructs. The WASP-expressing vectors used in the nonclinical studies to support the Biologics License Application (BLA) for Etuvetidigene autotemcel are included below (Table 1):

Table 1. Lentivirus vectors containing WAS transgene used in nonclinical development

(b) (4)

In vitro studies were conducted using CD34+ HSPCs or cell lines derived from WAS patients (intended clinical source cells) transduced with an indicated WAS LVV construct from Table 1. Likewise, for the in vivo studies, surrogate Lin- BM cells isolated from WASP knockout (WKO) mice were transduced with an indicated WAS LVV constructs from Table 1. The murine Lin- BM cells are functionally equivalent to the human CD34+ cells as they are enriched for HSPCs and avoid immune reactions to human cells in WKO mice and therefore are analogous to the autologous cell drug product, Etuvetidigene autotemcel (U Modlich, 2009). The transduction efficiency of the WAS LVV in murine Lin- BM cells (L Dupre, 2006) was also comparable to WAS patient CD34+ cells (S Scaramuzza, 2013).

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of Etuvetidigene autotemcel in pediatric WAS subjects.

In Vitro Studies

Study Number	Publication	Citation
1	Lentiviral vectors targeting WASP expression to hematopoietic cells, efficiently transduce and correct cells from WAS patients	S Charrier, 2007
2	Lentiviral Vector-Mediated Gene Transfer in T cells from Wiskott-Aldrich Syndrome Patients Leads to Functional Correction	L Dupre, 2004
3	Hematopoietic-Specific Lentiviral Vectors Circumvent Cellular Toxicity Due to Ectopic Expression of Wiskott-Aldrich Syndrome Protein	M Toscano, 2008

Note: Study Nos. 1 – 3 are briefly summarized in this review memo under ‘Overview of In Vitro Studies’.

In Vivo Studies

Study Number	Publication	Citation
4	Lentiviral-mediated gene therapy leads to improvement of B-cell functionality in a murine model of Wiskott-Aldrich syndrome	M Bosticardo, 2011
5	Dendritic cell functional improvement in a preclinical model of lentiviral-mediated gene therapy for Wiskott-Aldrich syndrome	M Catucci, 2012
6	A lentiviral vector encoding the human Wiskott-Aldrich syndrome protein corrects immune and cytoskeletal defects in WASP knockout mice	S Charrier, 2005
7	Efficacy of Gene Therapy for Wiskott-Aldrich Syndrome Using a WAS Promoter/cDNA-Containing Lentiviral Vector and Nonlethal Irradiation	L Dupre, 2006
8	Evidence for Long-Term Efficacy and Safety of Gene Therapy for Wiskott-Aldrich Syndrome in Preclinical Models	F Marangoni, 2009
9	Validation of a mutated PRE sequence allowing high and sustained transgene expression while abrogating WHV-X protein synthesis: application to the gene therapy of WAS	M Zanta-Boussif, 2009

Note: Study Nos. 4 – 9 are briefly summarized in this review memo under ‘Overview of In Vivo Studies.’

Overview of Pharmacology Studies

Overview of In Vitro Studies

The in vitro studies were conducted to evaluate the transduction efficiency and functional outcome in human WAS-deficient B cells, T cells, DCs, and CD34+ HSPCs following ex vivo LVV GT using different internal promoters: 1) WAS w0.5kb; 2) WAS w1.6kb; 3) Phosphoglycerate kinase 1 (PGK; pW); 4) Elongation factor 1 alpha (EF1; eW); 5) Spleen Focus-Forming Virus (SFFV; sW) (Table 1).

Key findings from Study 1 - 3 demonstrated that:

- LVV transduced B cells from WAS-deficient patients showed comparable WASP expression to healthy donor (HD) B cells.
- LVV transduced T cells from WAS-deficient patients demonstrated ameliorated T cell receptor (TCR)-driven proliferation and IL-2 production. Long-term culture of LVV transduced T cells also showed a selective survival advantage as compared to untransduced (UT) WAS-deficient T cells.

- LVV transduced DCs from WAS-deficient patients showed similar WASP expression, podosome structures and cytoskeletal organization (i.e., central F-actin core and peripheral ring of vinculin) as compared to HD DCs.
- WAS-deficient CD34+ cells transduced with the WAS w1.6kb LVV showed no detrimental effect on cell growth or survival and the appearance and size of colony-forming units (CFUs) in culture appeared normal when compared to HD CD34+ cells. Short- or long-term hematopoietic differentiation potential into myeloid, erythroid, and megakaryocyte lineages also was not adversely impacted.
- Although transcriptionally weaker (i.e., vector copy number [VCN] of ~1-2 copies/cell) than the constitutive, ubiquitously active promoters (i.e., PGK, EF1, SFFV), the endogenous WAS promoters (w0.5kb or w1.6kb) restored physiological levels of WASP in hematopoietic lineages without overexpression even at the highest multiplicity of infection (MOI = 100).

Reviewer comment:

Intracellular WASP is tightly regulated and not readily overexpressed due to the high specificity of the w1.6W vector using the endogenous WAS promoter and required co-expression with the WASP-interacting protein (WIP). The results from these studies using the endogenous WAS w1.6W LVV (b) (4) -grade) were used to identify the lead candidate for the clinical study.

Overview of In Vivo Studies

Mouse models/strains:

- The C57BL/6 (BL6) WKO mice were generated by deleting a large genomic region from intron 4 to exon 11 of the WAS gene (J Zhang, 1999). These mice exhibit mild thrombocytopenia with normal sized platelets, reduced monocyte counts, modest granulocytosis, and B cell lymphopenia as compared to wild type (WT) BL6 mice. BL6 WKO mice have a normal lifespan with no apparent defect in thymopoiesis but display a severe defect in T cell activation following TCR stimulation. The BL6 WKO mice require sublethal irradiation conditioning for transduced cell engraftment and were selected to evaluate the long-term safety and activity of LVV-mediated GT due to the low incidence of spontaneous tumors and long lifespan.
- Similarly, the 129 WKO mice (on a SV129 background) exhibit decreased thymocyte, peripheral B and T lymphocyte, and platelet counts as compared to WT 129 mice and develop a chronic colitis phenotype associated with abnormal T cell function (S Snapper, 1998). The 129 WKO mice require lethal irradiation conditioning and recapitulate key features of human WAS. The 129 WKO mice have a shorter lifespan as compared to the BL6 WKO mice.

Drug product used for the in vivo studies:

Surrogate Lin- BM cells transduced with the WAS LVV (w1.6W (b) (4)) were used as the test article (Table 1). Lin- BM cells without LVV transduction or Lin- BM cells transduced with an LVV expressing (b) (4) were used as

negative controls. Lin⁻ BM cells isolated from WT mice (i.e., BL6 or 129 background strain) were used as the positive control.

Key findings from Study 4 - 9 demonstrated that:

BL6 WKO mice findings:

- At 12 months, WKO mice present with B cell lymphopenia, thrombocytopenia, reduced monocyte counts, and modest granulocytosis. WKO GT BMT mice showed improved B cell, platelet, and granulocyte counts that were similar to WKO WT BMT mice.
- WKO mice that received WAS LVV transduced Lin⁻ BM cells showed high and stable engraftment (after 2 – 4 months) with restored WASP expression in CD45⁺ cells, B cells, T cells (CD4 and CD8 T cells), myeloid cells, and DCs in all lymphoid tissues as well as greater B cell and T cell survival and lymphocyte reconstitution over time (L Westerberg, 2008). The proportion of donor cells was >76% in the BM and spleen. Although the VCN was low (1-2 copies/cell), it was sufficient to restore WASP expression using the endogenous WAS promoter.
- WKO GT BMT mice showed an increase in WASP-expressing B cell subsets at all stages of maturity in the BM, peritoneal cavity, and spleen, and there was a selective advantage for B cells in the splenic marginal zone and peritoneal B1 cell subsets. WKO GT BMT mice exhibited an ameliorated autoimmune phenotype with a reduced frequency of mice expressing anti-double-stranded DNA (dsDNA) antibodies or were of a lower titer than the WKO UT BMT mice. B cells isolated from WKO GT BMT mice also showed improved immunoglobulin M (IgM) responses following pneumococcal vaccination.
- Bone marrow DCs (BMDs) isolated from WKO GT BMT mice showed improved phagocytosis, chemotaxis, and podosome formation that was comparable to WKO WT BMT mice. (b) (4) BMDs isolated from WKO GT BMT mice also demonstrated improved T cell priming responses with greater (b) (4)- and interferon- γ (IFN- γ)-positive T cell percentages and increased T cell proliferation as compared to the WKO UT BMT mice.
- Splenic T cells isolated from WKO GT BMT mice showed increased antibody-dependent proliferation, cytokine expression (i.e., IL-2, IFN- γ , tumor necrosis factor- α [TNF- α], IL-4, and IL-10), and F-actin polarization at the immunological synapse.
- WKO GT BMT mice experienced no deleterious effect on overall survival. Following 12 months post-transplantation, the tumor incidence in the WKO GT BMT mice was comparable to the control groups and were primarily of non-hematopoietic origin. Lymphomas were observed in 4 mice in the WKO GT BMT group and in 2 mice in the WKO UT BMT group. However, in all cases, real-time quantitative polymerase chain reaction (RT-qPCR) analysis confirmed that the lymphomas were of host origin and lacked LV integrations, which excluded the potential role of the GT contributing to the hematopoietic malignancies.

129 WKO mice findings:

- WKO mice develop an ulcerative colitis phenotype with associated diarrhea, thickening of the gut wall and mucosa with crypt hyperplasia, and a mixed infiltrate of lymphocytes and neutrophils within the lamina propria. WKO GT BMT mice showed >80% donor cell engraftment and expressed WASP in all hematopoietic cell lineages in the BM and spleen. The highest level of expression was noted in the splenic B cells and T cells, suggesting a selective advantage of the transduced cells to migrate to or expand in secondary lymphoid organs.
- WKO GT BMT mice showed improved survival as compared to WKO ^{(b) (4)} BMT mice, albeit less than the WKO WT BMT mice.
- Likewise, WKO GT BMT mice also showed an ameliorated phenotype with only focal or mild defects persisting in the gut histology. This effect was consistent with the correction of WASP expression in T cells associated with normalization of helper T and regulatory T cell function, which is often dysregulated in WAS patients and contributes to the development of autoimmune colitis (S Snapper, 1998).
- Primary WKO GT BMT mice with the highest donor chimerism and/or VCN were selected for secondary BMT into recipient WKO mice. Primary Lin⁻ BM cells engrafted in secondary WKO recipient mice and similarly showed multilineage hematopoietic cell reconstitution in all groups. Thymic lymphomas observed in 3 secondary WKO GT BMT mice were similarly of host origin and did not contain LV integrations.

Reviewer Comments:

- *Comparison of myeloablative conditioning regimens found the less aggressive sublethal irradiation protocol (used in BL6 WKO mice) to be sufficient to support long-term engraftment of the WAS LVV-transduced Lin⁻ BM cells. This suggested that more mild conditioning regimens may be suitable for use in WAS patients receiving GT.*
- *Both the w0.5W and w1.6W WAS LVVs were able to restore T cell activation and podosome formation in DCs following GT BMT in WAS-deficient mice.*
- *An in vivo enrichment for both B and T lymphocytes expressing WASP over time was observed following GT BMT in recipient WAS-deficient mice.*
- *None of the primary grafted animals developed donor-derived, LV-transduced hematopoietic tumors up to 12 months post-transplantation. Likewise, following the 10-month secondary experiment, WAS w1.6W LVV transduction of the primary Lin⁻ BM cells also did not lead to tumorigenesis in secondary BMT recipient WAS-deficient mice.*

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies were conducted for Etuvetidigene autotemcel and are not required for this drug product class (i.e., genetically modified CD34⁺ HSPCs). Due to the ex vivo transduction of target HSPCs, inadvertent expression in non-target tissues (e.g., heart, central nervous system [CNS], lungs) was considered unlikely and Etuvetidigene autotemcel is not anticipated to effect proteins capable of impacting the activity of CNS, cardiovascular (CV) or respiratory systems. Moreover, attempts to overexpress WASP were unsuccessful and adverse effects at low VCN are unlikely to have functional consequences.

PHARMACOKINETIC STUDIES

Summary List of Pharmacokinetics Studies

As Etuvetidigene autotemcel is an autologous, HSPC-based GT drug product, standard Absorption, Distribution, Metabolism, Excretion (ADME) and pharmacokinetic (PK) studies do not apply and were not conducted. However, the following cell fate studies to address survival/engraftment, distribution, and cellular differentiation were conducted because transduced CD34+ cells engraft into the BM and are a source of circulating blood cells that also contribute to resident immune cells in hematopoietic tissues (e.g., spleen, lymph nodes, thymus, liver).

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
10	Pilot Biodistribution study of human CD34+ cells transduced with (b) (4) in pre-conditioned NSG mice	TM019
11	Validation of cell density and viability determination	TV011
12	Validation of Flow Cytometry Stainings	TV018
13	Quantification of the number of integrated Lentiviral Vector copies in murine genomic DNA by Quantitative PCR analysis	TV022
14	Correction of Report TV026 (non-GLP): Validation of the method to quantify the number of integrated Lentiviral Vector copies in human genomic DNA by Quantitative PCR analysis (Amended Report)	TV026
15	Biodistribution study of human CD34+ cells transduced with GMP grade WASP LV in conditioned Rag2-/-IL2r gamma chain-/- mice	2017N340701_00
16	In vitro study on HSPCs transduced with WASP LV	592290
17	Preclinical Safety and Efficacy of Human CD34+ Cells Transduced With Lentiviral Vector for the Treatment of Wiskott-Aldrich Syndrome (S Scaramuzza 2013)	N/A
18	GSK2696274: Comparability Study of Cryopreserved and Fresh Formulation of Lentiviral Vector-Transduced CD34+ Cells Transplanted into NSG Mice	TM033

Note: The in vivo Study No. 10-18 (excluding Study No. 15) were not included in this memo as they were part of drug product and model optimization/development and are not directly relevant for the assessment of safety and activity of Etuvetidigene autotemcel. Study No. 10 was not included in this memo as it was primarily a pilot study for optimizing CD34+ HSC engraftment in non-obese diabetic severe combined immunodeficiency IL-2 receptor common gamma chain (NSG) mice and did not use the intended WAS LVV clinical drug product. Study No. 11 – 14 were not included in this memo as they primarily involved method validation. Study No. 16 was not included in this memo as it was primarily a comparability study using different HSPC sources and transduction protocols using the Good Manufacturing Practice (GMP)-grade WAS LVV (w1.6kb promoter). Study No. 18 was not included in this memo as it was primarily an evaluation of engraftment and hematopoietic reconstitution in NSG mice comparing fresh versus cryopreserved CD34+ cells following LVV transduction but did not use the intended WAS LVV clinical drug product.

Mouse models/strains:

Rag2^{-/-}IL2rg^{-/-} immunodeficient mice were used for the following studies due to their permissiveness to human HSPC engraftment, multilineage differentiation and long-term survival (E Traggiai, 2004). To achieve greater engraftment of human HSPCs into Rag2^{-/-}IL2rg^{-/-} mice, the neonatal mice were conditioned with a sub-lethal dose of total body irradiation (300 + 250 radiation absorbed dose [RAD]) to sufficiently deplete the bone marrow compartment.

Overview of Pharmacokinetic Studies**Study #15**

Report Number		2017N340701 00
Date Report Signed		09-Sep-2018
Title		Biodistribution study of human CD34+ cells transduced with GMP grade WASP LV in conditioned Rag2 ^{-/-} IL2r gamma chain ^{-/-} mice
GLP Status		No
Testing Facility		HSR-TIGET Ospedale San Raffaele Via Olgettina 58 20132 Milan, Italy
Objective(s)		To evaluate the ability of human hematopoietic stem/progenitor cells (HSPCs) to engraft and normally differentiate.
Study Animals	Strain/Breed	Rag2 ^{-/-} IL2rg ^{-/-} mice
	Species	(b) (4)
	Age	3-4 days old
	Body Weight	Not recorded
	#/sex/group	Please refer to Table 2 below.
	Total #	62
Test Article(s)		<p>Umbilical Cord Blood (UCB) derived HD CD34+ cells transduced at 100 MOI (2-hit protocol) with the clinical grade WASP LVV (Test Item, TI).</p> <p><i>Note: In previous studies, the applicant was able to achieve greater engraftment in Rag2^{-/-}IL2rg^{-/-} mice transplanted with CD34+ cells isolated from human UCB as compared to BM or mobilized peripheral blood (mPB) cells (WA Noort, 2001). Therefore, CD34+ cells from UCB instead of another HSPC source were used in this study to allow a more reproducible monitoring of the transplanted cell distribution.</i></p> <p>CD34+ cells isolated from HDs were used due to ethical limitations of WAS patient sourcing. Similar differentiation/growth and hematopoietic colony forming potential between WAS-deficient and HD cells was previously demonstrated in vitro following WAS LVV transduction (S Scaramuzza, 2013).</p>
Control Article(s)		<ol style="list-style-type: none"> 1. UCB-derived CD34+ cells cultured in the presence of cytokines but without transduction (Control Item untransduced, CI UT) 2. UCB-derived CD34+ cells not manipulated (Control Item Unmanipulated, CI UM)
Route of Administration		IV administration

Description of the Model and Transplant Procedure	On day -1 to day +14, prophylactic gentamicin sulfate (0.3 mg/mL) was included in the drinking water. Neonatal Rag2 ^{-/-} IL2rg ^{-/-} mice underwent total body irradiation with a standard sublethal dose of 550 RAD administered in two doses (300 + 250 RAD) given at a 3-hour interval to promote HSPC engraftment. Mice were then transplanted with UCB CD34+ cells (0.3 x 10 ⁶ cells/mouse) of either the test item or control items.
Dosing Regimen	Single administration
Randomization	No
Description of Masking	No masking
Scheduled Sacrifice Time Points	Mice were euthanized at 8 weeks

Study Groups and Dose Levels:

Table 2. Experimental Groups

Group	Condition	No. of Transplanted Mice	No. of Dead Mice	No. of Analyzed Mice	Sex
(b) (4), (b) (6)	TI	6	2	4	4 F
	TI	4	4	0	-
	CI UM	5	2	3	1 F / 2 M
	TI	6	4	2	2 M
	TI	5	1	4	3 F / 1 M
	CI UT	2	0	2	2 F
	CI UM	3	1	2	2 M
	TI	13	0	13	5 F / 8 M
	CI UT	6	0	6	3 F / 3 M
	CI UT	7	4	3	3 F
	CI UM	5	2	3	3 M

*Test item and control items were frozen and thawed before administration.

Note: At the time of transplantation (3-4 days old), it was too early to determine the sex of the mice. The sex of the mice was determined at week 7 when peripheral blood (PB) was collected; however, a number of mice died before sex determination.

Key Evaluations and Assessments:

- A viability check was conducted at least 3x/week. No body weights were recorded during the study. Deaths observed before day 28 post-transplantation were considered related to the conditioning, acute radiation toxicity, and/or transplantation procedure. Mice found dead during this period were not processed and carcasses were not retained.

- At week 7, PB samples were collected to measure human cell engraftment (i.e., CD34, CD45) by fluorescence-activated cell sorting (FACS) analysis and the percentage of different myeloid and lymphoid lineages (CD3, CD4, CD8, CD13, CD15, and CD19). Engraftment and distribution of the TI and CIs were similarly evaluated by FACS analysis in hematopoietic organs (i.e., bone marrow, spleen, thymus, and liver) following euthanasia.
- VCN was assessed by qPCR in hematopoietic organs and LV integration was assessed by B2 Short Interspersed Nuclear Element (B2-SINE) qPCR in hematopoietic and non-hematopoietic organs (i.e., brain and testis) to distinguish between LV integration in murine cells versus human cells.
- The presence of replication competent lentivirus (RCL) was assessed in a mouse that tested positive for human immunodeficiency virus type 1 (HIV-1) Group-specific antigen (Gag)-p24 capsid protein after receiving the TI.
- To assess the potential mobilization or vector shedding to bystander cells, C8166-45 cells permissive to viral infection were co-cultured with transduced CD34+ cells for 28 days. Following co-culture, an enzyme-linked immunosorbent assay (ELISA) was conducted on culture supernatant to evaluate the presence of HIV-1 Gag-p24 protein. DNA from cultured cells was also extracted and tested for the presence of vesicular stomatitis virus glycoprotein G (VSV-G) DNA by qPCR.

Key Results:

- A total of 20 premature deaths occurred within the first two weeks post-transplantation due to the toxicity of the conditioning regimen or the transplant procedure; however, comparable survival rates were observed in all groups.
- No statistically significant differences were observed between groups in human CD45+ cell engraftment levels evaluated at the end of the study. The transplanted cells were able to differentiate into both lymphoid and myeloid lineages in all test organs. The greater proportion of human cells in the PB, BM, and spleen was represented by the B cell (CD19+) population. The thymus showed a normal pattern of thymopoiesis and intra-thymic maturation of human T cells including CD3, CD4, and CD8 T cell subpopulations.
- Similar VCN values were observed in terminally differentiated progeny as compared to the pre-infusion sample, indicating that the administered HSPCs possessed long-term repopulation and multilineage differentiation potential in all hematopoietic organs. WAS LVV transduced cells were present in all hematopoietic tissues including the BM, thymus, spleen, and liver; however, high variability was observed between mice and specific organs reflecting the polyclonal nature of the TI engraftment.
- No evidence of bystander transduction in non-hematopoietic tissues (i.e., brain and testis) or germline transmission was observed, whereas the LV was observed in hematopoietic tissue compartments. Likewise, in order to assess the potential for mobilization or vector shedding to bystander cells, B2-SINE PCR was conducted on DNA extracted from tissue collected from transplanted mice to distinguish between LV integrated in murine cells from human cells. No evidence of vector integration in murine cells was observed including the mouse that was positive for the HIV-1 Gag-p24 protein.

- In addition to excluding the generation of RCL, HIV-1 Gag-p24 protein was also measured in mouse plasma. All mice tested were negative with the exception of one mouse in the (b) (4), (b) (6) TI group that showed a low level of HIV-1 Gag-p24 protein. More detailed molecular analyses were performed both on cells archived from this mice and cells cultured starting from the infused TI, which indicated the presence of gag-pol packaging plasmid sequences, but not HIV-1 long terminal repeats (LTR), in the mouse BM and in the absence of RCL. These findings suggested the occurrence of rare plasmid integration as a consequence of packaging plasmid carry-over in the LVV used for transduction. This event was not considered to be associated with any adverse pathology or possible biological risk to patients.

Reviewer comments:

- *The GMP WAS LVV (clinical grade) was used for this nonclinical study; however, human CD34+ cells were isolated from UCB rather than mPB to achieve higher engraftment efficiency in recipient Rag2^{-/-}IL2rg^{-/-} mice. CD34+ cells isolated from mPB intended to be used for the human clinical drug product would be anticipated to have similar engraftment efficiency as well as safety and activity as CD34+ cells isolated from UCB.*
- *The detection of HIV gag sequences in the BM of one mouse together with the evidence of HIV-1 p24 Gag antigen in the plasma raised the possibility that a RCL event may have occurred. However, the safety feature of the third-generation packaging system combined with a SIN transfer vector used in this study made it unlikely that a productive viral recombinant was generated during vector production or cell transduction. No evidence of RCL was detected in mouse tissues. The most likely interpretation of the findings is the occurrence of a stable transfection of the packaging plasmid in a rare cell engrafted in the mouse, which is possible given that the plasmids used for transient transfection of the vector-producer cells represent a major contaminant of the final vector product, even if the downstream purification removed almost 99.9% of this input. This event was not associated with pathology because it did not represent RCL and did not lead to vector mobilization because the Gag-containing particles released from the cells would lack an envelope to make them infectious as well as a viral ribonucleic acid (RNA), as the transduced SIN vector would be resistant to packaging and mobilization.*

Study #17

Overview of peer-reviewed publication (Scaramuzza et al.)

Nonclinical studies were conducted to optimize CD34+ cell transduction, isolated from HD and WAS patient samples, using a clinical grade WAS LVV (w1.6W). WAS LVV transduced CD34+ cells were characterized both in vitro and following hematopoietic engraftment in Rag2^{-/-}IL2rg^{-/-} mice via insertion site analysis (ISA) for oncogenesis risk assessment.

Note: More detailed description of the biodistribution study conducted in Rag2^{-/-}IL2rg^{-/-} mice is described in Study #15.

Key Evaluations and Assessments:

- HD mPB- or BM-derived CD34+ cells were purchased from Lonza (Basel, Switzerland) or were isolated from HD UCB and WAS patients' BM cells obtained after written informed consent according to ethical procedures with approval of the San Raffaele Institute Bioethical Committee. Following pre-stimulation, CD34+ cells were transduced with the w1.6W LV at up to 100 MOI for one- or two-hit transduction. For the 2-hit protocol, a wash period of 10-12 hours was included between vector exposures. Following w1.6W LVV transduction, the CD34+ cells were analyzed by flow cytometry for CD34 and CD45 cell surface marker expression and plated for colony-forming unit cells (CFU-C) and Long-Term Culture-Initiating Cells (LTC-IC).
- The integration profile of the w1.6W LVV and clonal repertoire of the transduced CD34+ cells isolated from a pool of 4 HDs (both BM and UCB) was assessed in vitro and in Rag2^{-/-}IL2rg^{-/-} mice via ligation-mediated/linear amplification-mediated PCR (LM/LAM-PCR) and pyrosequencing.

Key Results:

- Two-hit exposure to the w1.6W LVV resulted in higher VCN as compared to one-hit exposure for both HD mPB and BM. Likewise, the w1.6W LVV transduced HD CD34+ cells showed a similar LTC-IC as compared to the UT CD34+ cells with no evidence of toxicity on the bulk culture or for clonogenic progenitors. Similarly, WAS patient CD34+ cells showed a higher VCN and gene transfer into progenitor cells as compared to both HD BM- and mPB- derived cells in all conditions. In WAS BM CD34+ cells, an overall reduced capacity for growth in culture when exposed to proliferation-inducing factors was observed as compared to HD CD34+ cells. Likewise, in WAS BM CD34+ cells, a reduced recovery of cells after thawing and a decreased growth capacity and number of colony-forming unit cells (CFU-Cs) was observed when compared to HD CD34+ cells. However, no marked difference was observed between transduced CD34+ cells and UT CD34+ cells in terms of growth, CD34 expression, total number of CFC colonies, and proportion of progenitors (erythroid, myeloid, or mixed colonies).
- The in vitro insertional profile was typical of LVVs with significant preference for transcriptional units (from 74.5 to 77.0% of integrations) as compared to in silico generated random insertions (n = 100,000; 40.7%) and was similarly maintained in vivo, 8 weeks after transduced cell infusion in Rag2^{-/-}IL2rg^{-/-} mice. The number of unique insertions showed a polyclonal repertoire in vitro both in human CD34+ cells from HD and WAS patients and following engraftment in Rag2^{-/-}IL2rg^{-/-} mice. By comparing the insertions in vivo in the BM and thymus from individual TI administered mice or in BM, thymus, and spleen from a pool of three mice, the applicant observed the presence of shared identical integrants among the different hematopoietic compartments analyzed. No enrichment or increased frequency of integration sites in proximity to genes involved in oncogenesis from the Ingenuity Pathway Analysis software (Ingenuity Systems) was observed in vivo as compared to random frequency (6.3% in both HD and WAS patients versus 16.1% in the random in silico insertion dataset).

Reviewer comments:

- *Analysis of vector integration in vitro and in vivo showed a strong bias of the WAS vector for coding regions without major preference for regions upstream of transcription start sites, a typical feature of LVVs and proto-oncogenes (C Cattaglio, 2007). The insertional profile was polyclonal in human CD34+ cells both in vitro and after in vivo engraftment in Rag2^{-/-}IL2rg^{-/-} mice, consistent with the WAS-LVV integration profile using surrogate Lin- BM murine cells with no evidence of clonal proliferation and thus a low potential risk of insertional oncogenesis.*

TOXICOLOGY STUDIES**Summary List of Toxicology Studies**

The following toxicology studies were conducted to evaluate the safety of Lin- BM cell transplantation in WAS-deficient mice as well as an in vitro study assessing the potential for bystander transduction following co-culture with human CD34+ cells transduced with the WAS LVV (w1.6W).

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
19	Biosafety of the vector and transgene in vivo	2017N342790
20	GSK2696275: In vitro characterization of cryopreserved and fresh WAS CD34+ cells derived from mobilized peripheral blood	TV034
21	Study on secondary transduction by HSPCs transduced with WASP LV	2017N340702_00

Note: Study No. 20 was not included in this memo as it was primarily a comparability study using fresh and cryopreserved WAS CD34+ HSPCs isolated from mPB.

Developmental and Reproductive Toxicology Studies:

DART studies were not conducted for this drug product type in line with international guidelines and due to the lack of concerning findings in reproductive tissues. The applicant showed that distribution to reproductive tissues was near or below the background threshold, indicative of a low risk of LV-mediated germline transmission to reproductive tissues as supported by previous publications (I Visigalli, 2016). Additionally, no human or LVV sequences were detected in non-hematopoietic tissues (i.e., brain and testes), confirming that no DART studies were considered warranted for Etuvetidigene autotemcel.

Genotoxicity Studies:

Study Number	Publication	Citation
22	Insertional Transformation of Hematopoietic Cells by Self-Inactivating Lentiviral and Gammaretroviral Vectors	U Modlich, 2009
23	A high throughput method for genome-wide analysis of retroviral integration	J Mantovani, 2006

Study Number	Publication	Citation
24	Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral integration	E Montini, 2006
25	The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy	E Montini, 2009

Note: Study No. 22 – 25 were not included in this memo because the cited publications do not involve the WAS LVV drug product and are not directly supportive of the safety of Etuvetidigene autotemcel.

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 the applicant showed no evidence of tumorigenicity using the in vitro immortalization (IVIM) assay (U Modlich, 2009) and for the in vivo studies performed with no preferential integration near proto-oncogenes as evaluated via ISA.

Overview of Toxicology Studies

Study #19

(b) (4)

2 pages have been determined to be not releasable: (b)(4)

(b) (4)

Study #21

Study objective: To evaluate the potential risk of secondary transduction due to the persistence of viral particles on the surface of human CD34+ cells (isolated from UCB or BM) transduced with the w1.6W LVV cultured in the presence of (b) (4).

Study design: The following in vitro culture conditions included: 1) (b) (4)

Results: The persistence of LV particles on the transduced HSPC surface was responsible for secondary transduction proportional to the HSPC:(b) (4) ratio (mean (b) (4) in CI 1 for the (b) (4) ratio, respectively) but was significantly lower than the VCN observed in (b) (4) cells (mean VCN^{(b) (4)}) directly transduced with the w1.6W WAS LVV. (b) (4)

with (b) (4) significantly reduced secondary transduction of (b) (4) cells (mean VCN (b) (4) for TI 1 at the (b) (4) ratio, respectively). This effect was abrogated following (b) (4) for CI 2 (mean VCN (b) (4) at the (b) (4) ratio, respectively).

Reviewer Comment:

WAS transduced HSPCs induced a low bystander transduction of (b) (4) cells; however, brief (b) (4) of transduced HSPCs with (b) (4) cells. This effect was (b) (4). Overall, these data suggest that in a clinical setting, in which WAS LVV transduced CD34+ cells are infused into the bloodstream of patients, the potential occurrence of vector shedding from transduced cells, and thus secondary transduction events is likely negligible.

APPLICANT'S PROPOSED LABEL

Section 8.1 Use in Specific Populations

- No edits were made to this section of the label.

Section 12.3

- References to nonclinical data were removed from this section of the label.

Section 13 Nonclinical Toxicology

- Subsection headings 13.1 and 13.2 were added and edits were made to remove any items that did not directly inform on the safe and effective use of Etuveditigene autotemcel.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies using analogous vector and cells did not identify any safety concerns for the clinical dosing, route of administration, and indication for use. The nonclinical data support approval of this licensing application.

KEY WORDS/TERMS

WASKYRA, Etuveditigene autotemcel, lentiviral vector, hematopoietic stem cells, Wiskott-Aldrich Syndrome

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