

**FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Therapeutic Products
Office of Pharmacology/Toxicology
Division Pharmacology/Toxicology 2
Pharmacology/Toxicology Branch 2**

I concur with this memo. S. Sanduja

I concur with this memo. S. Tomar

BLA NUMBER:	STN #125758.000
DATE RECEIVED BY CBER:	07/19/2023
DATE REVIEW COMPLETED:	12/28/2024
PRODUCT:	Lenmeldy (atidarsagene autotemcel)
APPLICANT:	Orchard Therapeutics (Europe) Limited
PROPOSED INDICATION:	Treatment of pediatric patients with pre-symptomatic late infantile (PSLI), pre-symptomatic early juvenile (PSEJ) or early symptomatic early juvenile (ESEJ) metachromatic leukodystrophy (MLD)
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EXECUTIVE SUMMARY:

OTL-200 (Lenmeldy or atidarsagene autotemcel) consists of autologous CD34+ hematopoietic stem and progenitor cells (HSPCs) transduced *ex vivo* with a replication-incompetent, self-inactivating lentiviral vector (LVV) encoding human arylsulfatase A (ARSA). OTL-200 is intended for the treatment of pediatric patients with pre-symptomatic late infantile (PSLI), pre-symptomatic early juvenile (PSEJ) or early symptomatic early juvenile (ESEJ) metachromatic leukodystrophy (MLD). OTL-200 is administered to the patient following a busulfan based conditioning regimen to aid engraftment in the hematopoietic compartment with the goal of production of functional ARSA protein leading to break down or preventing the accumulation of

harmful levels of sulfatides involved in disease progression. The minimum recommended dose level of OTL-200 is $\times 10^6$ CD34+ cells/kilogram (kg) as a single-dose intravenous (IV) infusion for autologous use only.

In vitro studies with murine Lin- HSPCs (mHSPCs) from ARSA knockout (As2^{-/-}) mice transduced with a lentiviral vector encoding the ARSA gene (ARSA LVV) demonstrated reconstitution of sulfatide metabolism by the cerebroside 3-sulfate assay and ARSA activity levels exceeding wild type cells as measured by p-nitrocatechol (p-NC) sulfate assay. *In vitro* studies with human bone marrow-derived CD34+ cells from healthy donors and a patient with MLD showed similar levels of transduction and reconstitution of ARSA activity and ARSA-LVV transduction did not affect clonogenic potential or differentiation capacity of the CD34+ cells.

In vivo pharmacology studies conducted in pre-symptomatic and symptomatic As2^{-/-} mice demonstrated long-term engraftment of ARSA LVV-transduced mHSPCs in the bone marrow (BM) following intravenous (IV) administration of 1×10^6 cells/mouse ($40\text{--}50 \times 10^6$ cells/kg) in irradiated mice. Restoration of ARSA activity was observed in the peripheral blood monocytes and liver in these animals and reached 10% of wild type levels in the brain. Improvement in motor conduction abnormalities, motor coordination by rotarod testing, and neuropathology were observed compared to mice receiving GFP LVV-transduced mHSPCs when evaluated between 4-12 months post-transplantation. Correction of the disease phenotype correlated with: 1) engraftment of mHSPCs and vector copy number (VCN) in the BM and 2) ARSA activity in blood and target tissues.

Biodistribution (BD) studies conducted in irradiated immunodeficient mice demonstrated long-term engraftment and multilineage differentiation of ARSA LVV-transduced hHSPCs. In addition to the bone marrow, spleen, thymus, and liver, transduced human cells were also detected at lower levels in the mouse brain, demonstrating migration of the transduced cells into target organs. Additionally, these studies showed that integrated LVV persists in the differentiated progeny of transduced hHSPCs and remains stably integrated within human cells.

The *in vivo* safety of single IV administration of ARSA LVV-transduced mHSPCs at 1×10^6 cells/mouse ($40\text{--}50 \times 10^6$ cells/kg) in irradiated As2^{-/-} mice was evaluated in a pivotal GLP study for up to 12 months post-transplant. No test article-related mortality, toxicity or hematologic malignancy was observed, with study findings primarily attributed to the As2^{-/-} phenotype, irradiation, and incidental age-related findings. No development of anti-ARSA antibodies or adverse neurobehavioral effects were observed in As2^{-/-} mice transplanted with ARSA LVV-transduced mHSPCs. Integration site analysis showed polyclonal reconstitution of cells with no evidence of preferential expansion of integration sites near proto-oncogenes. A higher incidence of hepatocellular tumors was reported in a preceding non-GLP safety study in As2^{-/-} mice receiving ARSA LVV- and mock-transduced mHSPCs. In that study, tumors were not detected in wild type mice, suggesting a possible effect of the genetic background and higher dose myeloablative total body irradiation used in that study.

Genotoxicity risk was assessed *in vitro* using hHSPCs derived from healthy donors and MLD patients and showed no effects of LVV transduction or ARSA overexpression on the

proliferation or differentiation of hHSPCs. No evidence of insertional mutagenesis of the LVV backbone in an *in vitro* immortalization (IVIM) assay and no increase in tumorigenesis *in vivo* in a tumor-prone mouse model were demonstrated using a LVV expressing GFP, which shares the same LVV backbone as that of ARSA LVV.

Carcinogenicity and developmental and reproductive toxicity studies were not conducted with OTL-200. These studies are not warranted based on the product characteristics and safety profile.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies identified in this submission. There are no outstanding requests for additional nonclinical data for evaluation of Lenmeldy. The nonclinical information provided in the BLA submission supports approval of the licensure application.

Formulation and Chemistry:

OTL-200 (Lenmeldy or atidarsagene autotemcel) consists of autologous CD34+ HSPCs transduced *ex vivo* with a replication-incompetent LVV encoding human ARSA. The minimum recommended dose level of OTL-200 is $(b) (4) \times 10^6$ CD34+ cells/kg. OTL-200 is intended to be supplied as a cryopreserved suspension for infusion, manufactured from cells collected from mobilized peripheral blood (PB). It is supplied in a 50 mL (nominal volume) (b) (4) infusion bag (or multiple bags) at a concentration of 2×10^6 viable cells per mL, in a volume of 10 to 20 mL of cryopreservation medium (5% DMSO, (b) (4)) per (b) (4) bag. Administration of OTL-200 is via intravenous infusion (IV) for autologous use only.

ARSA LVV is a human immunodeficiency virus type 1 (HIV-1)-based self-inactivating (SIN), replication-incompetent vector pseudotyped (b) (4) and contains an expression cassette for the *ARSA* transgene under the control of the human PGK promoter. The PGK promoter was selected (b) (4)

The ARSA LVV also contains a (b) (4)

The structure of the ARSA LVV construct is shown below in Figure 1.

(b) (4)

Abbreviations

ALD	Adrenoleukodystrophy
ARSA	Arylsulfatase A
ARSA-HA LVV	Lentiviral vector expressing arylsulfatase A tagged with a C-terminal HA epitope
ARSA LVV	Lentiviral vector expressing arylsulfatase A
BM	Bone marrow
CB	Cord blood
CD	Cluster of differentiation
CIS	Common insertion site
CNS	Central nervous system
GFP	Green fluorescent protein
GFP LVV	Lentiviral vector expressing green fluorescent protein
GLP	Good laboratory practice
HSPC	Hematopoietic stem and progenitor cell
IVIM	<i>In vitro</i> immortalization
Lin-	Lineage negative
LV	Lentivirus
LVV	Lentiviral vector
mL	Milliliters
MLD	Metachromatic leukodystrophy
MOI	Multiplicity of Infection
(b) (4)	
(b) (4)	
PBMC	Peripheral blood mononuclear cell
p-NC	P-nitrocatechol
(b) (4)	
SIN	Self-inactivating
SR-TIGET	San Raffaele Telethon Institute for Gene Therapy

VCN	Vector copy number
WPRE	Woodchuck hepatitis virus posttranscriptional regulatory element
WT	Wild type

Related File(s)

IND#26917: Autologous CD34+ Hematopoietic Stem Cells Transduced with Lentiviral Vector Expressing Human Arylsulfatase A gene for the treatment of Metachromatic Leukodystrophy (MLD); Orchard Therapeutics (Europe) Ltd; Active.

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INTRODUCTION

Metachromatic leukodystrophy (MLD) is a rare autosomal recessive lysosomal storage disorder caused by biallelic pathogenic mutations in the arylsulfatase A (ARSA) gene that result in deficiency of the encoded lysosomal ARSA enzyme. The ARSA enzyme is essential for the metabolism of sulfatides and its deficiency results in accumulation of the undegraded substrates in the lysosomes of oligodendrocytes, microglia, certain neurons of the CNS, Schwann cells, and macrophages in the PNS. This leads to progressive demyelination, microglial damage, neurodegeneration and subsequent loss of motor and cognitive functions, and early death, especially in patients with early symptom onset (< 7 years of age).

Based on the age of symptom onset, MLD is classified into late infantile (LI), age of onset ≤ 30 months; early juvenile (EJ), between 30 months and 7 years; late juvenile (LJ) between 7 and 16 years; and adult, with age of onset ≥ 17 years. There are currently no approved therapies in the United States for the treatment of MLD. The standard of care for patients with MLD is supportive or palliative care, which does not alter the progressive and fatal course of the disease.

OTL-200 consists of autologous CD34+ HSPCs transduced with ARSA LVV encoding the human ARSA enzyme, which is intended to break down or prevent the harmful build-up of sulfatides. After OTL-200 infusion, transduced hHSPCs engraft and repopulate the hematopoietic compartment with the goal of a sub-population of the transduced hHSPCs engrafting in the brain and differentiating into resident microglia and perivascular macrophages capable of secreting functional ARSA enzyme. These pharmacologic effects resulting from durable expression of ARSA are expected to reduce the demyelination and neurodegeneration and improve clinical manifestations of MLD.

NONCLINICAL STUDIES

Reviewer's Notes:

- The applicant has used the terms Lenmeldy, OTL-200 and atidarsagene autotemcel interchangeably in the nonclinical study reports. Throughout the 'Nonclinical Studies' Section of this memo, the product will be referred to as ARSA LVV-transduced murine HSPCs (mHSPCs) or human HSPCs (hHSPCs), as applicable.
- The LVV vector used in Nonclinical Studies 1 and 2 summarized in this memo contained the (b) (4) WPRE element; the remaining nonclinical studies used the clinical LVV with the (b) (4) [REDACTED]. Additionally, some of the studies conducted in the mouse MLD model and ARSA transgenic mice (ARSA Tg) used the LVV with (b) (4) WPRE but with the transgene containing a C-terminal hemagglutinin (HA) epitope tag. This epitope tagged-ARSA LVV was designated ARSA-HA LVV and was used for immunolocalization studies. A GFP LVV with the transfer vector backbone identical to ARSA LVV and expressing green fluorescent protein (GFP), was also used in several studies. The nonclinical product used is specified under each study.
- The *in vivo* activity of OTL-200 was supported by studies with mHSPCs transduced with ARSA LVV and injected into ARSA knockout mice ($As2^{-/-}$), a murine model of MLD. Per the applicant, this model was generated by targeted disruption of murine ARSA and is characterized by slow and progressive disease manifestations in the nervous system such as motor coordination impairment, delayed motor conduction in the CNS and PNS, neuronal degeneration and widespread storage of undegraded sulfatides. The storage pattern of undegraded sulfatides and neuropathology findings (vacuolated cells present in the white matter of cerebellum and in the optic and sciatic nerves) described in $As2^{-/-}$ mice resemble those of MLD patients. However, compared to MLD patients, $As2^{-/-}$ mice

have a mild phenotype as they do not develop the widespread demyelination and severe symptoms seen in humans and have a normal life span.

- Per the applicant, obtaining hHSPCs from MLD patients for use in all nonclinical studies was not feasible due to ethical considerations. Therefore, lineage negative (Lin-) mHSPCs (derived from the BM of As2^{-/-} mice) and healthy hHSPCs (derived from umbilical cord blood or BM) transduced with ARSA LVV were used for the majority of the nonclinical studies. Due to a lower transduction efficiency observed with hHSPCs compared to mHSPCs, a transduction protocol of (b) (4) at MOI of (b) (4) was selected for the clinical product compared to (b) (4) at MOI of (b) (4) for the murine surrogate product. A comparison of transduced hHSPCs from healthy donors and an MLD patient showed no differences in proliferation or hematopoietic colony forming potential *in vitro*. Therefore, the use of ARSA LVV-transduced healthy donor HSPCs was acceptable to generate data to support the development of OTL-200.

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of OTL-200 to treat the proposed clinical indication.

Study Number	Study Title / Publication Citation	Report Number
1	Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells; J Clin Invest. 2004 Apr;113(8):1118-29. doi: 10.1172/JCI19205.	Biffi, 2004
2	Gene therapy of metachromatic leukodystrophy reverses neurological damage and deficits in mice; J Clin Invest. 2006 Nov;116(11):3070-82. doi: 10.1172/JCI28873.	Biffi, 2006
3	Characterization of human CD34 ⁺ cells transduced with pre-GMP grade ARSALV (<i>in vitro</i> and <i>in vivo</i>).	2017N330774

* All these studies were conducted at SR-TIGET, Milan, Italy, and results from Study #1 and #2 were reported as publications.

Overview of Pharmacology Studies

Study #1

Biffi. A et al. Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells; J Clin Invest. 2004

This publication evaluated the fate of ARSA LVV-transduced MLD mHSPCs and prevention of MLD progression following transplantation in presymptomatic As2^{-/-} MLD mice. Transduction of Lin- mHSPCs with ARSA LVV resulted in reconstitution of ARSA-dependent sulfatide metabolism in cells by cerebroside 3-sulfidate assay. Additionally, the p-NC assay demonstrated ARSA activity in transduced cells to be 5-10-fold higher than the WT controls.

Six weeks old MLD mice were administered ARSA LVV-transduced mHSPCs (1×10^6 cells/mouse) via the tail vein following total body irradiation. The peripheral blood mononuclear cells (PBMCs) of the transplanted MLD mice showed reconstitution of ARSA-dependent sulfatide metabolism by LRh-sulfatide reduction and detection of galactosylceramide (LRh-GalCer) seven months post-transplant, with ARSA-specific activity quantified as four to eight-fold higher than that observed in wild type (WT) mice. These data show complete reconstitution of ARSA enzyme activity in the hematopoietic compartment of ARSA LVV-transduced mHSPCs transplanted mice.

Prevention of motor conduction impairment was also observed at 4-, 7- and 11-months post-transplant compared to mice receiving GFP-transduced mHSPCs. Slight improvements in these parameters were also observed compared to WT mHSPC-transplanted mice, suggesting potential therapeutic benefit with overexpression of ARSA. Twelve-month old mice demonstrated improvement in motor learning and coordination deficits by rotarod testing compared to age-matched controls and WT HSC-transplanted mice. Histopathological analysis at 12 months of age demonstrated a marked reduction of sulfatide-containing metachromatic granules in the white matter of the brain and cerebellum of ARSA LVV transplanted mice, as compared to age-matched untreated or GFP LVV mHPSC recipient mice.

Reviewer Comments:

- The publication also evaluated administration of mHSPCs transduced with GFP LVV under the control of the PGK promoter in C57BL/6 mice to assess cell marking, biodistribution, and hematopoietic reconstitution via secondary transplant. These studies are not described in further detail since the studies evaluated the GFP LVV.
- Based on the available information regarding the $As2^{-/-}$ mouse model, they reflect an early stage of the human disease¹ but represent a suitable model for studying the effect of ARSA LVV-based gene therapy.
- The results from this study support the rationale for the transplantation of ARSA LVV-transduced autologous HSPCs and that overexpression of ARSA may provide therapeutic activity in preventing disease progression in MLD.

Study #2

Biffi. A et al. Gene therapy of metachromatic leukodystrophy reverses neurological damage and deficits in mice; J Clin Invest. 2006.

This publication evaluated the potential of ARSA-HA LVV-transduced mHSPCs to correct the disease phenotype in symptomatic six-month-old female $As2^{-/-}$ MLD mice. Mice were administered ARSA-HA LVV-transduced mHSPCs (1×10^6 cells/mouse) via the tail vein

¹ Hess B et.al, Phenotype of arylsulfatase A-deficient mice: relationship to human metachromatic leukodystrophy, PNAS 1996

following total body irradiation. Average vector copy numbers ranged from 4.1 to 7.8. ARSA activity evaluated by cerebroside 3-sulfidate assay using PBMCs of transplanted mice (6 months post-transplant) indicated a 3- to 5- fold increase compared to WT levels. In the liver, ARSA activity was 2.1-fold higher compared to WT mice at 12 months post-transplant. Reconstitution of sulfatide metabolism was also detected in the brain at levels corresponding to an average 10% of WT activity in pooled samples from ARSA-HA LVV mHSPC-administered mice. Mice receiving ARSA-HA LVV-transduced cells showed correction of neurological deficits measured by neurophysiological evaluation, electroneurographic recordings, rotarod test, and reduction of neuropathological damage at 6 months post-transplant. Additionally, the improvement in the MLD phenotype was increased with higher levels of ARSA overexpression in the mHSPC-derived microglia. Widespread ARSA-HA distribution was detected in these cells throughout the CNS, along with detection of ARSA-HA in endogenous neurons and Purkinje cells, suggesting possible cross-correction through uptake of functional enzyme secreted by the transduced cells. These results indicate that the ARSA-LVV-transduced HSPCs can mediate production of ARSA in the CNS, allowing for correction of markers of disease in the brain.

Reviewer Comment:

- The data from this study indicates that administration of ARSA LVV-transduced HSPCs can lead to CNS distribution of the ARSA enzyme and supports the rationale for administration of ARSA LVV-transduced HSPCs for the treatment of patients with MLD.

Study #3

Characterization of human CD34+ cells transduced with pre-GMP grade ARSA LV (in vitro and in vivo) (Non-GLP; Study Report # 2017N330774; HSR-TIGET, Italy)

This study evaluated (b) (4) different protocols for the large-scale manufacturing of ARSA LVV-transduced hHSPCs derived from bone marrow of healthy donors and a patient with MLD and assessed transduction efficiency, expansion, *in vitro* activity, and *in vivo* engraftment and hematopoietic reconstitution.

(b) (4)

Reviewer's Notes:

- This report is a summary of *in vitro* and *in vivo* data generated from various studies conducted at TIGET between 2007 and 2008. The final, signed version of the report was received by GSK from TIGET in April 2016.
- The (b) (4) mouse model was selected for its permissiveness to engraftment and long-term survival of hHSPCs.

The results from these experiments showed Protocol E had the highest transduction efficiency and ARSA expression in BM-derived hHSPCs *in vitro*, with maintenance of adequate cell numbers, CD34 expression and clonogenic potential. Similar transduction efficiency and ARSA activity was observed between the healthy donor cells and MLD patient-derived cells. The *in vivo* data demonstrated that ARSA LVV-transduced hHSPCs engrafted in the (b) (4) neonatal mice and differentiated into multiple lineages. No significant differences in engraftment and differentiation were observed between hHSPCs transduced using the (b) (4) transduction protocols. The VCN in the differentiated progeny was comparable to the VCN in the pre-transplant hHSPCs.

Reviewer's Comments:

- The report was prepared retrospectively by GSK and most of the data are generated from screening studies evaluating different transduction protocols and not all of the data were verifiable based on the review findings in the report.
- The (b) (4) transduction protocols appear to result in similar levels of engraftment and differentiation *in vivo*. Per the applicant, the Protocol E was selected for clinical manufacturing since the highest transduction efficiency was achieved with the (b) (4) step followed by (b) (4) of transduction at a MOI of (b) (4).

PHARMACOKINETIC STUDIES (Biodistribution)

Summary List of Pharmacokinetics Studies

The following biodistribution (BD) studies were conducted.

Study Number	Study Title / Publication Citation	Report Number
4	Biodistribution study of human CD34+ cells transduced with GMP grade ARSA LV in conditioned (b) (4) mice.	2017N327519
5	Comparability study of cryopreserved and fresh formulations of lentiviral vector transduced CD34+ cells transplanted into (b) (4) mice.	2016N302792
6	Brain conditioning is instrumental for successful microglia reconstitution following hematopoietic stem cell transplantation; Proc Natl Acad Sci U S A. 2012 Sep 11;109(37): 15018-23.doi: 10.1073/pnas.1205858109. Epub 2012 Aug 23.	Capotondo, 2012

Overview of Pharmacokinetic Studies

Reviewer's Notes:

- Study #6 is considered a supplementary study since it evaluates conditioning regimens for successful microglia reconstitution post HSC transplant. The findings are briefly summarized at the end of this section.
- In Module 4, Section 4.2.2.1 of the BLA, the applicant provided analytical methods and validation reports for: i) cell density and viability of primary murine cells (mouse BM cell from (b) (4) /background) and a human suspension cell line ((b) (4)); ii) staining of murine lineage, splenocytes and peripheral blood cell surface markers using (b) (4) ; iii) VCN; and iv) human ARSA activity measured by (b) (4) assay. The reports and analytical methods used are acceptable.

Study #4

Biodistribution study of human CD34+ cells transduced with GMP grade ARSA LV in conditioned (b) (4) mice (Non-GLP; Study Report # 2017N327519; HSR-TIGET, Italy)²

Objective:

To assess BD of untransduced (UT) or ARSA LVV-transduced hHSPCs following transplantation into sub-lethally irradiated neonatal (b) (4) mice.

Materials and Methods:

- *Test article:* UCB-derived hHSPCs transduced with ARSA LVV. Three different donor lots were tested.
- *Control articles:* Unmanipulated (UM) UCB-derived hHSPCs (thawed immediately before transplantation) and untransduced (UT) UCB-derived hHSPCs (mock transduced and cultured similar to the test article for (b) (4) prior to transplantation).
- Irradiated animals with no test article administration were also used as controls.
- On Day 1, a dose level of $1.88\text{--}2.5 \times 10^5$ cells/10 μ L/mouse of test or control (UT or UM) article was administered IV into the temporal vein for the study groups.
- BD and engraftment were evaluated in blood, BM, spleen, thymus, liver, brain, and gonads (testes only) via (b) (4) -based detection of hCD45+ cells at Week 6 and Week 8 (termination).
- Engraftment and VCN was evaluated in BM, spleen, thymus, liver, and gonads (testes only) via (b) (4) using (b) (4) at Week 8.
- Replication competent LV in plasma was evaluated via detection of HIV-1 (b) (4) at Week 8.

(b) (4)

Results:

Mice (n)	Organ	% CD45+ cells	LVV (copies)	Human cells(copies)	VCN	Human DNA (ng)	Murine DNA (ng)	Human cell engraftment	(b) (4)
22	Thymus	67	9x10 ³	5x10 ³	3.7	-	-	-	Negative
	BM	34	4x10 ³	2x10 ³	3.4	91.17	41.50	41.55	
	Spleen	11	2x10 ³	9x10 ³	3.4	-	-	-	
	Liver	22	8x10 ³	4x10 ³	3.0	-	-	-	
	Brain	-	14	9	3.7	0.06	112.22	1.72	
	Gonads (testes)	-	9	4	2.3	0.01	24.50	0.06	

* -: not evaluated

Table 1: BD of ARSA LVV-transduced hHSPCs in immunodeficient mice.

- Engraftment of hHSPCs was observed in all the groups receiving hHSPCs. No differences were observed in the BD of control and test articles.
- Persistence of the integrated LVV was observed in the terminally differentiated progeny of the ARSA LVV-transduced hHSPCs. VCN in the progeny cells was similar to the VCN in the pre-transplant hHSPCs.
- (b) (4) results also showed engraftment of ARSA LVV-transduced hHSPCs in the brain of transplanted mice; low levels of the LVV were detected in the testes.
- No LVV was detected in the blood.

Study #5

Report Number		2016N302792
Date Report Signed		07/07/2017
Title		Comparability study of cryopreserved and fresh formulations of lentiviral vector transduced CD34+ cells transplanted into (b) (4) mice
GLP Status		Yes Italian GLP and OECD Principles of GLP
Testing Facility		GLP SR-TIGET, Milan, Italy
Objective(s)		To compare the cell engraftment and differentiation into mature progeny for the cryopreserved and fresh formulations of OTL-200.
Study Animals	Strain/Breed	(b) (4)
	Species	Mouse
	Age	8 weeks
	Body Weight	16.7-25.9 grams
	#/sex/group	Refer to the table in the Study Groups and Dose Levels section. Only females were included in the study. Reviewer's Note: ➤ The applicant did not provide a reason for including only females in this study.
	Total #	124

Test Article(s)	1. hHSPCs transduced with ARSA LVV - fresh formulation (referred as 'Reference Item'(RI)) 2. hHSPCs transduced with ARSA LVV - cryopreserved formulation (referred as 'Test item' (TI)) Reviewer's Note: ➤ The product used commercially will be a cryopreserved formulation.			
Control Article(s)	None			
Route of Administration	IV injection			
Description of the Disease/Injury Model and Implant Procedure	Mice were sub-lethally irradiated ((b) (4)) on Day 1 and the test articles were administered within 6 hours of irradiation.			
Study Groups and Dose Levels	Group	Transplanted cells	Dose level (cells/mouse)	Number of mice
	1	None	-	8
	2	RI	5×10^5	12
	3	RI	2×10^5	12
	5	RI	0.5×10^5	12
	6	RI	0.2×10^5	12
	7	None	-	8
	8	TI	5×10^5	12
	9	TI	2×10^5	12
	10	TI	1×10^5	12
	11	TI	0.5×10^5	12
	12	TI	0.2×10^5	12
Dosing Regimen	Single IV infusion			
Randomization	Yes; Not specified			
Description of Masking	Not provided			
Scheduled Sacrifice Time Points	20 weeks post-dose			

Key Evaluations and Assessments:

- Mortality- Daily
- Clinical observations- Daily
- Body weight- Weekly
- Engraftment in blood, BM, and spleen via ((b) (4)) -based detection of hCD45+ cells- Weeks 8, 19 and 20

- Differentiation into multilineage cells in blood, BM, and spleen using (b) (4) - based detection of CD19, CD3 and CD13 expression on hCD45+ cells- Weeks 8, 19 and 20
- VCN and engraftment in BM, spleen and brain via (b) (4) using (b) (4) - Week 20
- Gross pathology and histopathology- Week 20

Key Results:

- No significant differences in survival, clinical signs, body weights or gross pathology were observed between the groups.
- Reduced size of ovaries observed in all female mice in both the TI and RI groups, regardless of the dose level of the test articles. Per the pathologist, this was an expected finding after exposure to irradiation.

Reviewer's Comment:

- This reviewer agrees with the pathologist's conclusion.

(b) (4)

Reviewer's Comment:

- Based on the results for hCD45+ cell engraftment, and VCN in the BM samples, this reviewer agrees with the study director's conclusion that the fresh and cryopreserved formulations of ARSA LVV-transduced hHSPCs are similar in their potential for engraftment and multilineage differentiation.

Study #6

Capotondo et.al. Brain conditioning is instrumental for successful microglia reconstitution following hematopoietic stem cell transplantation. Proc Natl Acad Sci USA. 2012

This publication evaluated the effect of the myeloablative conditioning regimen (total body irradiation or busulfan) on the migration of transplanted mHSPCs to the CNS and on donor-derived microglial reconstitution in WT and As2^{-/-} mice, at various timepoints after mHSPC transplantation. The results showed infiltration of the transplanted mHSPCs in the brain, independent of the conditioning regimen or the disease status. However, the effect was more pronounced with busulfan and As2^{-/-} mice were more sensitive to the conditioning effect than WT mice. Busulfan conditioning ablated myeloid precursors in the brain, which allowed turnover of endogenous microglia with the donor-derived microglia. The turnover of endogenous microglia was mediated by local proliferation of early donor cell immigrants rather than entrance of mature cells from the circulation.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following studies were conducted to evaluate the safety of ARSA LVV-transduced mHSPCs following administration in mice.

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
7	Toxicology and tumorigenicity study in ARSA KO mice treated with ARSA ^{-/-} HSPCs transduced with ARSA.LV vector.	2016N302788
8	Long-term assessment of mice transplanted with ARSA-LV transduced and control HSPCs.	2017N330775
	• GSK(b) (4) - Long-term Assessment of Mice Transplanted with ARSA-LV Transduced and Control HSPCs (Dataset 1).	2018N364205
	• GSK(b) (4) - Long-term Assessment of Mice Transplanted with ARSA-LV Transduced and Control HSPCs (Dataset 2).	2018N364207
9	Testing of different pre-transplant conditioning regimens in MLD Mice.	2015N232105
10	Safety of arylsulfatase A overexpression for gene therapy of metachromatic leukodystrophy; Hum Gene Ther. 2007 Sep;18(9):82136. doi:10.1089/hum.2007.048.	Capotondo, 2007

Reviewer's Note:

- Study #10 is considered a supportive toxicology study since it did not use the clinical vector construct with (b) (4) WPRE. The findings are briefly summarized at the end of this section.

Overview of Toxicology Studies

Study #7

Report Number		2016N302788		
Date Report Signed		02/03/2017		
Title		Toxicology and tumorigenicity study in ARSA KO mice treated with ARSA ^{-/-} HSPCs transduced with ARSA.LV Vector		
GLP Status		Yes Per Italian GLP and OECD Principles of GLP		
Testing Facility		GLP SR-TIGET		
Objective(s)		To assess the toxicity and tumorigenic potential of ARSA ^{-/-} HSPCs transduced with ARSA LVV (GSK (b) (4)) in As2 ^{-/-} mice, the mouse model of MLD		
Study Animals	Strain/Breed	(b) (4) As2 ^{-/-}		
	Species	Mouse		
	Age	6-8 weeks old		
	Body Weight	Male: 15.7-24.9 grams; Female: 15-20.7 grams		
	#/sex/group	Refer to table in the Study Groups and Dose Levels section		
	Total #	109		
Test Article(s)		mHSPCs transduced with ARSA LVV (Lot No. GSK (b) (4))		
Control Article(s)		mHSPCs mock transduced		
Route of Administration		IV		
Description of the Disease Model		The As2 ^{-/-} mouse model on a C57Bl6 background is an MLD disease model recapitulating the biochemical and some pathological features of the MLD disease. Transplantation of HSPCs into As2 ^{-/-} mice requires conditioning with a standard sublethal dose of total body irradiation to deplete endogenous BM cells. In this study mice received myeloablative total body irradiation twice, at (b) (4), 2 hours apart (total dose (b) (4)). Mice in the MLD (UT) group were not irradiated.		
Study Groups and Dose Levels		Group	Dose levels	Mice (n)
		MLD (UT)	-	18 (M), 16(F)
		MLD (Test)	1±0.2x10 ⁶ cells/mouse	18 (M), 20(F)
		MLD (Mock)	1±0.2x10 ⁶ cells/mouse	20 (M), 17(F)
Dosing Regimen		Single IV infusion		
Randomization		No; Not specified		
Description of Masking		Not provided		
Scheduled Sacrifice Time Points		52 weeks post-dose		

Key Evaluations and Assessments:

- Mortality- Daily
- Clinical observations- Daily
- Neurobehavioral assessment- Weeks 8 and 40
- Body weight- Weekly
- Food consumption- Weekly

- Clonogenicity of PBMCs via (b) (4) assay- Weeks 10 and 30
- Hematology- Weeks 10, 30 and 52
- Engraftment in PB, BM, spleen, liver, and thymus via (b) (4) -based detection of donor mCD45+ cells- Weeks 10 and 52
- VCN in BM, spleen, thymus, and liver via (b) (4) using (b) (4) - Week 52
- ARSA activity- Week 52
- Anti-ARSA antibody detection- Week 52
- Replication competent LV via detection of (b) (4) - Week 52
- Gross pathology and histopathology- Week 52

Key Results:

- Several deaths were reported before Day 28 [MLD (UT)- 1, MLD (Test)- 1, MLD (Mock)- 5] and after Day 29 [MLD (UT)- 3, MLD (Test)- 20 (5M, 15 F), MLD (Mock)- 17 (4M, 13 F)]. MLD (Test) and MLD (Mock) groups showed similar incidence of unscheduled deaths, with more deaths among females than males.

Reviewer's Note:

- Per the study director, deaths before Day 28 were a result of conditioning and occurred only in males. Increased mortality observed after Day 29 in females were due to neoplastic disease, renal injury, and lymphoid depletion with a concomitant adenoma of duodenum and were attributed to host response to the conditioning regimen, disease progression and age due to the long study duration.

Reviewer's Comment:

- This reviewer agrees with the study director's conclusion.
- No test article-related adverse findings were observed for all the parameters assessed.
- Engraftment in lympho-hematopoietic tissues (PB, BM, spleen, and thymus) was higher compared to the liver.
- No sex-dependent differences were observed in VCN in the PB.
- MLD (Test) mice showed reconstitution of ARSA activity in the BM (mean 39.34 nmol/h.mg) and liver (mean 13.06 nmol/h.mg) in comparison to undetectable activity in MLD (UT) mice.
- No anti-human ARSA antibodies were detected.
- No lentivirus was detected in the serum of either MLD (Test) or MLD (Mock) groups.
- Vector integration site analysis showed random integration with no preferential bias for integration in the promoter regions.
- Histopathological changes associated with the MLD phenotype were significantly reduced in incidence and severity in the MLD (Test) group as compared to MLD (UT) and MLD (Mock) groups.
- Per the study director, total body irradiation resulted in pathology findings in various organs in MLD (Test) and MLD (Mock) groups, consistent with reported literature. Renal

injury (observed in all females and majority of males), focal cortical hyperplasia, hypertrophy of adrenal glands, and stomach glandular dilatation was also observed in these groups. There was no effect of the test article on the incidence of proliferative changes. Hemo-lymphoproliferative disorders were observed at similar incidences in all groups (two lymphomas in MLD (UT), one lymphoma and one granulocytic leukemia in MLD (Test) and one histiocytic sarcoma in MLD (Mock)). Other proliferative changes (such as liver carcinoma, small intestine adenocarcinomas, small intestine adenoma, skin fibrosarcoma, lung bronchiolo-alveolar adenoma, tubular renal adenoma) were observed in irradiated groups only [MLD (Test) and MLD (Mock)] and have been reported to occur spontaneously in aged mice. Hepatocarcinoma was found only in one male mouse transplanted with ARSA LVV-transduced cells and in one male dosed with mock-transduced cells.

Reviewers' Comments:

- This reviewer agrees with the study director's conclusion. Findings from this study appear to primarily be attributed to the irradiation procedure, MLD phenotype, or age-related changes.
- The higher incidence of liver tumors detected in Study 8 (reviewed below) was not observed in this study. Per the applicant, the myeloablative conditioning regimen of two exposures at (b) (4), 2 hours apart (total (b) (4)) was lower compared to the single exposure at (b) (4) used in the previous study for adult mice, which may have contributed to the differences observed between the studies. Additionally, the applicant notes that it is likely that some genetic drift might have occurred in the colony of As2^{-/-} mice used in the GLP study as the mice were further rederived and intercrossed in the 6 years between the two studies.

Study #8

Long-term assessment of mice transplanted with ARSA-LV transduced and control HSPCs (Non-GLP; Study Report # 2017N330775; HSR-TIGET, Italy)

Reviewer's Note:

- This report includes combined data from studies conducted by the applicant under two study reports (Report Nos. 2018N364205 and 2018N364207). Report No. 2018N364205 contains data for studies conducted between October 2006 and July 2008 in As2^{-/-} mice, while Report No. 2018N364207 contains data for studies conducted between March 2008 and September 2009 in adult WT, neonatal WT or As2^{-/-} mice. The salient findings from each study report are summarized below.

Report No. 2018N364205:

This study evaluated the toxicity and tumorigenic potential of ARSA LVV-transduced mHSPCs (test article, Lot No. GSK (b) (4)) when transplanted in young adult As2^{-/-} mice (5-8 weeks old) following irradiation ((b) (4)) for adults and (b) (4) for neonates). Murine HSPCs transduced

with ARSA LVV, GFP LVV, or mock-transduced cells were transplanted into sub- female As2^{-/-} mice at approximately 1x10⁶ cells/mouse. Mice were observed for 10-12 months after transplantation. Groups of irradiated age-matched As2^{-/-} mice without mHSPC transplantation were included as controls for pathological examination.

Results:

- Transplantation of the test article had no effect on mortality.
- Mice administered the test article showed reconstitution of ARSA activity in PB at levels ranging between 0.4 to 7.7-fold the average values reported for WT mice.
- No antibody response against ARSA was observed.
- VCN in the BM ranged from 2.8 to 10.9, which demonstrates a robust engraftment of the test article.
- No signs of toxicity, hematological abnormalities, or development of hematopoietic tumors due to the test article were observed. A slightly higher incidence of hepatocellular tumors (mostly adenoma) was observed in As2^{-/-} mice transplanted with the test article (5/25) in comparison with controls (1/14 in mock-transduced and 1/23 in unmanipulated As2^{-/-} mice). Per the study director, this finding was considered likely to be related to the myeloablative conditioning regimen and the genetic background of mice. Based on the detection of hepatic lesions, a separate study was conducted in adult and neonatal mice to investigate the observed liver changes (see summary of Report No. 2018N364207 below).

Report No. 2018N364207:

Murine HSPCs, transduced with ARSA LVV, GFP LVV or unmanipulated were transplanted into sub-lethally irradiated adult WT (1 to 2 months old), neonatal WT or As2^{-/-} mice (7 to 9 days old) at approximately 1x10⁶ cells/mouse. Irradiated, untransplanted mice were added as irradiation controls. Total body irradiation was carried out as a single dose ((b) (4)) for neonates) and mice were observed for 8-10 months after transplantation.

Results:

- Transplantation of the test article did not have any effect on mortality.
- Mice administered the test article showed reconstitution of ARSA activity in PB with levels 3 to 23-fold higher than the WT mice.
- VCN ranged between 10.2 and 32.7.
- Transplantation of the test article did not cause toxicity, hematological abnormalities, or development of hematopoietic tumors in adult WT, neonatal WT, or As2^{-/-} mice.
- Liver abnormalities were seen in As2^{-/-} mice, including in the irradiation control group and in As2^{-/-} mice transplanted with unmanipulated WT mHSPCs, but were not observed in WT recipient mice. Proliferative/neoplastic lesions in the liver were detected in As2^{-/-} mice receiving LVV ARSA-transduced MLD mHSPCs (10/17), unmanipulated WT HSPCs (4/15), LVV GFP transduced MLD HSPCs, and (1/3) irradiation controls. Per the study report, the occurrence of liver lesions in As2^{-/-} mice, and not in WT mice that share the same genetic background, suggested that the irradiation procedure and/or ARSA deficiency might influence the development of liver neoplasia in these mice.

Reviewer's Comment:

- This reviewer agrees with the study director's conclusion that the irradiation procedure or the As2^{-/-} knockout in the mice may have contributed to the development of hepatocarcinoma and liver lesions observed in the As2^{-/-} mice.

Study #9

(b) (4)

Study #10

Capotondo. A et al. Safety of arylsulfatase A overexpression for gene therapy of metachromatic leukodystrophy; Hum Gene Ther. 2007.

This publication evaluated the impact of LVV-mediated ARSA overexpression on the long-term viability and function of human HSPCs. Safety of overexpression of ARSA was also evaluated in transgenic mice carrying multiple copies of the LVV sequence in the genome. hHSPCs were isolated and transduced with ARSA LVV and were used for clonogenic assays and *in vivo* study in 3 days old Rag2^{-/-}Il2r gamma chain^{-/-} mice. Post irradiation, these mice were administered transduced hHSPCs via IV injection into the temporal vein. ARSA overexpression in hHSPCs did not impair their clonogenic and multilineage differentiation capacities in clonogenic assays and in the neonatal hematochimeric mice, respectively. In transgenic mice carrying multiple copies of the LVV sequence in their genome and with LVV-mediated ARSA overexpression up

to 15-fold above the normal range in all tissues, mice showed maintenance of immune functions of the hematopoietic compartment, and normal behavioral and cognitive functions. The activity of sulfatases other than ARSA that also depend on the same activator, sulfatase-modifying factor-1 (SUMF1), was not affected in ARSA-overexpressing hHSPCs *in vitro* or in transgenic mice. Overall, these data support the safety of ARSA overexpression at levels needed for the correction of MLD phenotype.

Genotoxicity Studies:

Study Number	Study Title / Publication Citation	Report Number
11	Lentiviral vector common integration sites in preclinical models and a clinical trial reflect a benign integration bias and not oncogenic selection; Blood. 2011 May 19;117(20):5332-9. doi: 10.1182/blood-2010-09-306761. Epub 2011 Mar 14.	Biffi, 2011
12	Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors; Mol Ther. 2009 Nov;17(11):1919-28. doi: 10.1038/mt.2009.179. Epub 2009 Aug 11.	Modlich, 2009a
13	Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration; Nat Biotechnol. 2006 Jun;24(6):687-96. doi: 10.1038/nbt1216. Epub 2006 May 28.	Montini, 2006

Study #11

Biffi et al. Lentiviral vector common integration sites in preclinical models and a clinical trial reflect a benign integration bias and not oncogenic selection (Gene Therapy. 2011)

This publication evaluated common insertion sites (CISs) for LVV integration in LVV-transduced hHSPCs engrafted in immunodeficient mice and compared it to the integration sites reported in patients' cells in an HSPC-based clinical trial for X-linked adrenoleukodystrophy (ALD). 85% of the CISs identified in the tissues of hHSPC-transplanted mice matched the CISs reported in patients with ALD that received LVV-transduced HSPCs. Most of the CISs in the murine and patient samples clustered in specific mega base-wide chromosomal regions. LVV integrations were widely distributed at and around CISs and these integrations were not enriched after transplantation into mice. Insertional mutagenesis was not observed either in the hHSPC-transplanted mice or during the 2-year follow-up of the ALD clinical trial. In contrast, cancer-triggering CISs reported in γ -retroviral vector (RVV)-based clinical trials typically target a single gene and are contained within a narrow genomic region.

Study #12

Modlich et al. Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors (Mol Ther. 2009)

This publication evaluated the risk for insertional mutagenesis by self-inactivating gammaretroviral vectors (SIN- γ -RVV) versus SIN-LVV. The study tested a panel of 13 different vectors using an *in vitro* immortalization (IVIM) assay capable of detecting cell

transformation induced by these vectors. LVV with their preferred integration in transcribed genes were less mutagenic than γ -RVV with their preference for integration next to transcriptional start sites and regulatory regions. Some SIN-LVVs also triggered cell transformation of hematopoietic cells depending on the type of internal enhancer–promoter used. Despite major differences in their integration mechanisms, both γ -RVV and LVV shared a common integration site in the first intron of the *Evi1* proto-oncogene. Thus, the mechanistic studies in this publication support the conclusion that activation of protooncogenes genes through insertion of a vector’s enhancer can cause transformation of hematopoietic cells and appropriate vector design can substantially lower the frequency of insertional oncogenesis.

Study #13

Montini et al. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration (Nature Biotechnology, 2006)

This publication evaluated the risk of insertional oncogenesis by RVV and LVV using HSPCs from *Cdkn2a*^{-/-} mice, which are highly susceptible to developing a variety of tumors. Vector integration sites and tumor development was compared between mice transplanted with RVV- or LVV-transduced HSPCs. RVV triggered dose-dependent acceleration of tumor onset, which was contingent on the activity of the integrated long terminal repeat (LTR). Insertions at oncogenes and genes involved in cell-cycle regulation were enriched in early-onset tumors. In contrast, LVV did not increase incidence of tumor development in *Cdkn2a*^{-/-} mice and there was no enrichment of transduced cells with specific integration sites despite high VCN and robust expression of LVV in all hematopoietic lineages. Thus, this publication demonstrated decreased mutagenic potential from integration of SIN-LVV.

APPLICANT’S PROPOSED LABEL

Sections 8 (‘Use in Specific Populations’) and 13 (Nonclinical Toxicology) are generally acceptable; minor edits will be recommended.

CONCLUSION OF NONCLINICAL STUDIES

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

KEY WORDS/TERMS

OTL-200, mice, LVV, engraftment, genotoxicity, HSPCs, hematopoietic, ARSA activity, MLD, transduction, integration, differentiation, VCN, irradiation, conditioning regimen.