15 October 2020
EMA/584450/2020
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Libmeldy

Usual common name: Autologous CD34+ cell encoding ARSA gene

Procedure No. EMEA/H/C/005321/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

%LV+	percentage of bone marrow-derived colonies harboring the lentiviral vector genome
AAA	anti-arylsulfatase A antibodies
AAV	Adeno-associated vector
ACP	Abnormal clonal proliferation
ADA-SCID	adenosine deaminase severe combined immunodeficiency
ADR	adverse drug reaction
AE	adverse event
AEMPS	Spanish Agency of Medicines and Medical Devices
aHUS	atypical hemolytic uremic syndrome
AIFA	Italian Medicines Agency
ALT	ALT Alanine aminotransferase
ANC	absolute neutrophil count
ANCOVA	analysis of covariance
ARSA	arylsulfatase A
AST	Aspartate aminotransferase
AUC	area under the curve
BAER	Brainstem auditory evoked responses
BM	bone marrow
CA	Competent authority
CAT	Committee for Advanced Therapy Medicinal Products
CDISC	Clinical Data Interchange Standards Consortium
cDNA	complementary deoxyribonucleic acid
CFH	complement factor H
CFR	Code of federal regulations
CFU-C	Colony-forming units in culture
CFU-GM	Colony-forming units in culture-granulocyte, monocyte
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CMV	Cytomegalovirus
CNS	central nervous system
CQA	critical quality attributes
CRF	Case report form
CSF	cerebrospinal fluid
CSR	clinical study report
CTA	Clinical trial application
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
CUP	compassionate use programme
CVC	central venous catheter
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DP	drug product
DQ	developmental quotient

DS	drug substance
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
EAP	Expanded Access Programme
EBV	Epstein-Barr virus
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	electronic case report form
EEG	Electroencephalogram
EJ	early juvenile
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ENG	electroneurography
ERT	enzyme replacement therapy
EU	European Union
FDA	Food and Drug Administration
GCP	good clinical practice
G-CSF	granulocyte colony stimulating factor
GMFC	gross motor function classification
GMFM	gross motor function measure
GSK	GlaxoSmithKline
GT	gene therapy
GVHD	graft versus host disease
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HGVS	Human Genome Variation Society
HE	Hospital Exemption
HIV	human immunodeficiency virus
HSC	haematopoietic stem cells
HSCT	haematopoietic stem cell transplantation
HSPC	haematopoietic stem and progenitor cells
ICF	Informed Consent Form
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IgE	immunoglobulin E
IQ	intelligence quotient
IS	Integration site
ITT	Intent-to-Treat
IV	intravenous
LI	Late infantile
LJ	Late juvenile
LLOQ	Lower limit of quantification
LS	least squares
LSD	lysosomal storage disease
LTR	long terminal repeat
LV	lentiviral vector
MAA	Marketing Authorisation Application

MAC	myeloablative conditioning
Max	Maximum
MAS	Matched Analysis Set
MCP	membrane cofactor protein
MEB	Medicines Evaluation Board
MedDRA	Medical Dictionary for Regulatory Activities
MEP	Motor evoked potentials
Min	Minimum
MLD	metachromatic leukodystrophy
MMRM	Mixed-model repeated measures
MNC	mononuclear cell
mPB	mobilised peripheral blood
MRI	magnetic resonance imaging
NBS	New Born Screening
NCI	National Cancer Institute
NCV	Nerve conduction velocity
NCE	Neurological clinical evaluation
NHx	natural history
OLTFU	Observational Long-Term Follow-Up
OS	overall survival
OSR	Ospedale San Raffaele
OTL	Orchard Therapeutics (Europe) Ltd.
PB	peripheral blood
PBMC	peripheral blood mononuclear cells
PBSC	peripheral blood stem cells
PCR	polymerase chain reaction
Pd	Pseudodeficiency
PDCO	Pediatric Committee
PEG	percutaneous endoscopic gastronomy
PI	Principal investigator
PIP	Pediatric Investigational Plan
PK	pharmacokinetic
PNS	peripheral nervous system
PT	preferred term
QC	quality control
QP	Qualified Person
RAP	Reporting and Analysis Plan
RCL	replication-competent lentivirus
RNA	ribonucleic acid
SAE	serious adverse event
SD	Standard deviation
SAWP	Scientific Advice Working Party
SCE	Summary of Clinical Efficacy
SMAC	sub-myeloablative conditioning
SMFS	severe motor impairment-free survival
SmPC	Summary of Product Characteristics

SMQ	Standard MedDRA qu
SOC	system organ class
SOP	Standard operating procedures
SR-TIGET	San Raffaele Telethon Institute for Gene Therapy
TCR	T cell receptor
TE	transfection efficiency
ULN	Upper limit of normal
US	United States
VCN	vector copy number
VEP	Visual evoked potentials
VOD	veno-occlusive disease
VSV	vesicular stomatitis virus
VSV-G	vesicular stomatitis virus glycoprotein
WAS	Wiskott-Aldrich Syndrome
WHO	World Health Organisation
WISC	Wechsler Intelligence Scale for Children
WPPSI	Wechsler Preschool and Primary Scale of Intelligence

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Orchard Therapeutics (Netherlands) B.V. [Orchard] submitted on 8 November 2019 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Libmeldy through the centralised procedure falling within the Article 3(1) and point 1a.

Libmeldy, was designated as an orphan medicinal product EU/3/07/446 on 13 April 2007 in the following indication: Treatment of metachromatic leukodystrophy.

The applicant applied for the following indication:

Treatment of metachromatic leukodystrophy (MLD) in patients from birth to before 17 years and in older patients for whom disease onset occurred before 17 years.

Treatment with Libmeldy should be performed before the disease enters its rapidly progressive phase.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Libmeldy as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/libmeldy

The legal basis for this application refers to:
Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0222/2016 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0222/2016 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to

the proposed indication.

New active Substance status

The applicant requested the active substance autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells transduced *ex vivo* using a lentiviral vector encoding the human arylsulfatase A gene contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Protocol assistance

The applicant received the following Protocol assistance for Libmeldy (previous names for the product were also Telethon002, GSK2696274, OTL-200) on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
23 April 2009	EMEA/H/SA/1224/1/2009/PA/PED/ADT/III	*Prof. Fernando de Andrés Trelles, Dr John Warren*
25 September 2014	EMEA/H/SA/1224/1/FU/1/2014/ADT/III	*Dr Mario Miguel Rosa, Prof. Fernando de Andrés Trelles*
23 April 2015	EMEA/H/SA/1224/1/FU/2/2015/PA/PED/ADT/II	*Dr Mario Miguel Rosa, Dr André Elferink*
28 April 2016	EMEA/H/SA/1224/1/FU/3/2016/PA/PED/ADT/I, EMEA/H/SA/1224/2/2016/PA/PED/ADT/I	*Dr Mario Miguel Rosa, Prof. Fernando de Andrés Trelles*
22 June 2017	EMEA/H/SA/1224/2/FU/1/2017/PA/PED/ADT/I	*Prof. Fernando de Andrés Trelles, Dr Jonathan Sisson*
22 February 2018	EMEA/H/SA/1224/1/FU/5/2017/PA/PED/ADT/II	*Dr Mair Powell, Dr André Elferink*

The Scientific Advices pertained to the following quality, non-clinical and clinical aspects:

- The proposed approach to process performance qualification (PPQ) and comparability for the vector manufacturing process, drug substance and drug product process; the proposed test methods for GSK3484865 (starting material), GSK2696274 (drug substance) and GSK2696274 Dispersion for Infusion (drug product), including the two stage drug product release strategy; definition of the active substance; the proposal of a two stage release strategy and the test methods for GSK2696274 (drug substance) and cryopreserved GSK2696274 Dispersion for Infusion (drug product); the proposed identity testing of GSK3484865 lentiviral vector; the strategy for PPQ to demonstrate commercial process consistency for the manufacture of drug substance and drug product; the proposed release strategy; sufficiency of the proposed design and number of stability

studies to support the proposed commercial cryopreserved product shelf life.

- The proposed comparability studies with the current fresh formulation and a new cryopreserved formulation and need of additional non-clinical studies prior to a later clinical trial with the cryopreserved formulation; sufficiency of the comparability exercise to conclude whether product manufactured using the proposed commercial manufacturing processes is comparable to that produced using the clinical production process.
- Appropriateness of the non-clinical package for MAA; adequacy of the biodistribution data to support the MAA; the design of the proposed non-clinical toxicity and tumorigenicity study.
- Whether it would be acceptable to submit the MAA with a 2 year (rather than 3 year) endpoint and without the final report of the GLP toxicity and tumorigenicity study.
- Design of the proposed phase I/II study, including patient selection, endpoints, sample size, early stopping rules/put on hold criteria; adequacy of the safety database for MAA; possibility of MAA submission based on 3 year follow-up efficacy and safety data in nine patients with late infantile and early juvenile MLD, who were treated in the asymptomatic or early symptomatic stages; whether treatment of all paediatric MLD patients (i.e. actual or predicted onset of symptoms at or below the age of 16 years, including asymptomatic LI and asymptomatic or early symptomatic juveniles) would be supported by an extrapolation strategy, based on efficacy in the youngest children with the most aggressive forms of the disease; whether the medical need in adults with MLD is separate and distinct from that in children and adolescents younger than 16 years, and thus supports limiting the target population to those with actual or predicted onset of symptoms under the age of 16; the proposed statistical analysis plan for the phase I/II study; the strategy for MAA submission for pre-symptomatic Late Infantile MLD; the revised MAA submission strategy, to file the cryopreserved formulation by first intent; appropriateness of the proposed patient population of predominantly pre-symptomatic Late Juvenile MLD and the enrolment criteria defined for Study 206790 to evaluate the safety and efficacy in Late Juvenile MLD; sufficiency of the endpoints selected for Study 206790 to evaluate the safety and efficacy in Late Juvenile MLD patients; the proposed approach for external control groups to contextualise the results of Study 206790; the statistical analysis plan and sample size proposed for Study 206790; sufficiency of the duration of Study 206790 and follow-up period for the proposed endpoints; adequacy of the clinical development programme to support approval in the treatment of all pre-symptomatic paediatric MLD patients (LI, EJ, LJ and Intermediate below 16 years of age).

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Carla Herberts Co-Rapporteur: Paolo Gasparini

CHMP Coordinator (Rapporteur): Hans Hillege CHMP Coordinator (Co-Rapporteur): Daniela Melchiorri (past); Armando Genazzani (current)

PRAC Rapporteur: Brigitte Keller-Stanislawski

The application was received by the EMA on	08 November 2019
Accelerated Assessment procedure was agreed-upon by CAT and CHMP on	11 October 2020
The procedure started on	28 November 2019
The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	17 February 2020
The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	17 February 2020
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	03 March 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	12 March 2020
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	20 March 2020
The applicant submitted the responses to the CAT consolidated List of Questions on	17 July 2020
The following GCP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GCP inspection at 2 sites (one investigator site in Italy and the sponsor site in the UK) between 27 January 2020 and 14 February 2020. The outcome of the inspection carried out was issued on:	18 March 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	31 August 2020
The Rapporteurs circulated the updated Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	02 September 2020
The CAT agreed on a list of outstanding issues in writing to be sent to the applicant on	11 September 2020
The applicant submitted the responses to the CAT List of Outstanding Issues on	17 September 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	30 September 2020

The outstanding issues were addressed by the applicant during an oral explanation before the CAT during the meeting on	7 October 2020
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Libmeldy on	9 October 2020
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Libmeldy on	15 October 2020

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Metachromatic leukodystrophy (MLD) is a rare autosomal recessive inherited lysosomal storage disorder caused by mutations in the Arylsulfatase A (ARSA) gene that results in deficiency of its corresponding enzyme.

The applicant initially sought approval of Libmeldy for the following indication:

"*treatment of metachromatic leukodystrophy (MLD) in patients from birth to before 17 years and in older patients for whom disease onset occurred before 17 years.*

Treatment should be performed before the disease enters its rapidly progressive phase"

During the assessment the indication was changed to the following:

Libmeldy is indicated for the of treatment metachromatic leukodystrophy (MLD) characterized by biallelic mutations in the arysulfatase A (ARSA) gene leading to a reduction of the ARSA enzymatic activity:

- in children with late infantile or early juvenile forms, without clinical manifestations of the disease,

- in children with the early juvenile form, with early clinical manifestations of the disease, who still have the ability to walk independently and before the onset of cognitive decline *(see section 5.1).*

2.1.2. Epidemiology

MLD is pan-ethnic, with affected patients described in several populations (including European, Japanese, Jewish, Lebanese, Muslim Arab, South African, Iranian, Indian, Polynesian, Algerian, Habbanite Jew, Navajo Indian, Alaskan Eskimo, and Christian Arab) (Von Figura, 2001).

A paucity of published MLD epidemiology data makes it difficult to accurately estimate the global prevalence/incidence of MLD; however, a systematic review of available literature has revealed approximately

1.1 cases (all MLD variants) per 100,000 livebirths in the European Union (EU) (Heim, 1997, Hult, 2014, Lugowska, 2011, Pinto, 2004, Poorthuis, 1999, Stellitano, 2016). This figure may be higher in areas or communities of the world in which consanguineous marriage is more common (Bindu, 2005, Harvey, 1993, Heinisch, 1995, Von Figura, 2001, Zlotogora, 1980). Furthermore, European studies suggest that approximately 40% to 60% of patients have the LI variant, 20% to 40% have the juvenile variant (early juvenile [EJ] + late juvenile [LJ]), and approximately 18% to 20% have an adult variant (Gieselmann, 2010, Gomez-Ospina, 2006, Heim, 1997, Ługowska, 2005, Poorthuis, 1999).

2.1.3. Biologic features

MLD is an autosomal recessive inherited lysosomal storage disorder caused by mutations in the ARSA gene that results in deficiency of its corresponding enzyme. ARSA breaks down cerebroside 3 sulfate (sulfatide), a major component of oligodendrocyte and Schwann cell myelin membrane in the central nervous system (CNS) and peripheral nervous system (PNS), respectively. ARSA deficiency results in accumulation of the undegraded substrate in lysosomes of oligodendrocytes, microglia, certain neurons of the CNS, Schwann cells and macrophages of the PNS, and other non-neural tissues (e.g., gallbladder, liver, pancreas, and kidneys). Accumulation in the nervous system, in turn, leads to microglial damage, progressive demyelination, neurodegeneration, and subsequent loss of motor and cognitive functions and early death, especially in patients with early disease onset (Bergner, 2019, Gieselmann, 2010, van Rappard, 2015). Therefore, ARSA deficiency, neuroinflammation, neurodegeneration and microglia activation as a result of sulfatide accumulation are key components of the common disease pathophysiology across the MLD clinical spectrum.

2.1.4. Clinical presentation

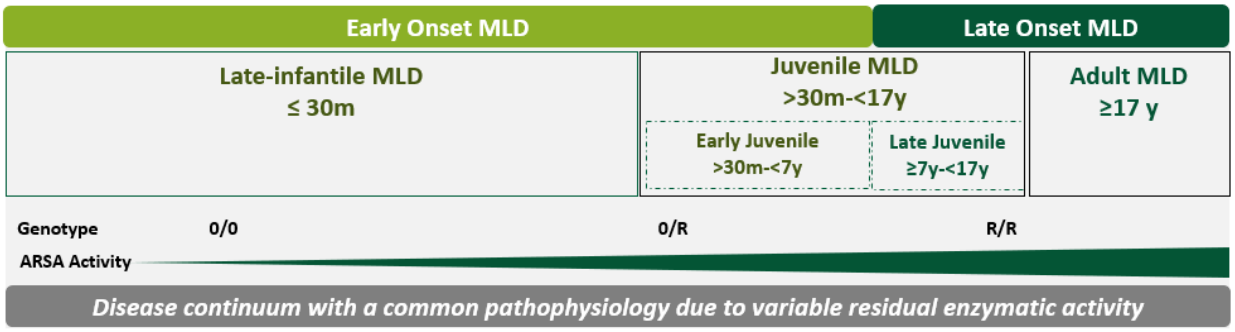
The MLD disease spectrum can present in a variety of clinical forms primarily based on the arbitrary criterion of age of onset of the first symptoms of the disease, rather than biological and clinical parameters which widely overlap across the disease spectrum. There is no universally accepted classification system for MLD phenotypes and at least three clinical forms of the disease are commonly described (LI-MLD, Juvenile MLD, and Adult MLD) (Kolodny, 1995, Von Figura, 2001). Despite this standard classification into different clinical phenotypes, it is well known that the underlying disease pathophysiology described above is common for all phenotypic forms of MLD. The arbitrary classification of MLD is particularly applicable to the stratification of juvenile forms into early and late juvenile.

Genetic mutations leading to MLD, can be functionally divided into 2 broad groups: null (0) alleles associated with no enzymatic activity and R alleles encoding for ARSA with some residual enzymatic activity (see Figure 1). The 3 most frequent variants reported to cause a partial or total disruption of the ARSA enzymatic activity and lead to MLD are the splice donor site variant “null allele” c.465+1G>A (traditionally named 459+1G>A), frequently found in LI-MLD patients and the missense “R allele” variants c.1283C>T (traditionally named 1277C>T) and c.542T>G (traditionally named 536T>G), usually found in association with a juvenile or adult phenotype. At least two hundred MLD-related ARSA mutations have been described, the frequency of which differs within ethnic groups (Cesani, 2016).

For illustrative purposes only, Figure 1 displays a simplified version of the relationship and boundaries between genotype/phenotype of MLD variants. The standard classifications of MLD variants are arbitrary due to the overlap in time course, symptomatology, age of onset, genotype, and progression rate between variants. Regardless of the clinical classification, the clinical course of the disease can be broadly divided into a pre-symptomatic stage with normal motor and cognitive development, followed by onset of first symptoms

and a period of developmental plateau, which is short in early onset forms and longer and more variable in late onset forms. The disease inevitably ends in a decerebrated state and eventually death for all phenotypic forms of the disease, although its course and duration are highly variable (Biffi, 2008a, Elgün, 2019, van Rappard, 2015).

Figure 1: Simplified MLD Genotype-Phenotype Relationship



ARSA= arylsulfatase A; MLD=metachromatic leukodystrophy; O=null allele; R=allele with residual enzyme activity

Patients who are clinically classified with the LI-MLD usually carry 2 null alleles (0/0 genotype) and hardly express any residual ARSA activity, resulting in symptoms manifestation before 30 months of age. LI MLD is the most prevalent MLD variant and the most aggressive form of the disease showing a highly predictable and severe disease course, characterised by progressive decline in motor and cognitive function and an early death (Gieselmann, 2010, van Rappard, 2015).

The phenotypic variability is particularly evident in the juvenile and adult variants that have at least one R allele, which results in some residual ARSA enzymatic activity to partially metabolise sulfatide and, thus, leads to a slower accumulation of undegraded substrate in the central and peripheral nervous systems (Rauschka, 2006, Sevin, 2007). The variability is observed in terms of clinical presentation, age at disease onset, and dynamics in the rate of disease progression (Biffi, 2008a, Lugowska, 2005, Polten, 1991).

In particular, patients who are affected by the Early Juvenile (EJ) MLD variant carry either 1 null allele and 1 residual allele (0/R genotype), or less frequently two residual alleles (R/R genotype), have symptom onset between the ages of 30 months and 6 years of age (before their 7th birthday), and tend to have slower and more variable initial disease progression.

Late Juvenile MLD variants (age/projected age at disease onset ≥7 years and <17 years) , on the other hand carry either two residual alleles (R/R genotype) or less frequently 1 null allele and 1 residual allele (0/R genotype) and predominantly develop cognitive and behavioral symptoms before or simultaneously with deterioration of gait and motor function (Biffi, 2008a, Gieselmann, 2010).

A study by Mahmood et al that was conducted via a retrospective analysis of MLD cases since 1921 showed that the 5-year survival after symptom onset was 25% and the 10-year survival was 0% for LI patients. However, since 1970, increased survival rates in a vegetative state have been observed, likely due to improvements in supportive care. For juvenile patients (mean age of diagnosis 10 years), 5- and 10-year survival rates were 70% and 44%, respectively (Mahmood, 2010).

2.1.5. Management

There is currently no curative treatment for MLD.

Available treatments only address the symptoms of the disease and none of them have proven to reverse the fatal outcome.

Allogeneic haematopoietic stem cell transplantation (HSCT) has been used for the treatment of MLD but results available so far have been inconsistent and associated with risks for serious complications, such as graft-rejection, graft versus host disease (GVHD) or complications derived from intense conditioning regimens. Given the more rapid progression in early onset MLD variants, the use of HSCT has been particularly limited to MLD patients with late-onset variants where, considering the associated risks of conditioning and those secondary to allogenic transplantation (GVHD, engraftment failures), the benefit risk profile remains to be determined.

Other investigational approaches are currently being tested in clinical trials such as intrathecal enzyme replacement therapy (ERT).

Also, a Chinese Phase I/II clinical trial (NCT03725670) using a self-inactivating lentiviral vector TYF-ARSA is ongoing; in this study, the investigational gene therapy is administered to MLD patients. Of note, a clinical trial evaluating the efficacy and safety of intracerebral in vivo gene therapy with adeno-associated vectors expressing ARSA (Clinical Trials.gov Identifier NCT01801709) has been recently discontinued (Schiller, 2019).

2.2. About the product

Libmeldy is an *ex vivo* genetically modified autologous $CD34^+$ haematopoietic stem and progenitor cell gene therapy. Autologous $CD34^+$ haematopoietic stem and progenitor cells (HSPCs) are collected from patient bone marrow (BM) harvest or from mobilised peripheral blood (mPB) and transduced with a lentiviral vector (ARSA LVV), which inserts one or more copies of the human ARSA complementary deoxyribonucleic acid (cDNA) into the cell's genome so that genetically modified cells become capable of expressing the functional ARSA enzyme. When administered to the patient following the administration of a myeloablative conditioning regimen, the genetically modified cells engraft and are able to repopulate the haematopoietic compartment. A subpopulation of the infused HSPCs and/or their myeloid progeny is able to migrate across the blood brain barrier to the brain and engraft as central nervous system (CNS) resident microglia and perivascular CNS macrophages as well as endoneural macrophages in the peripheral nervous system (PNS). These genetically modified cells can produce and secrete supraphysiological levels of the ARSA enzyme, which can be taken up by surrounding cells, a process known as cross-correction, and used to break down or prevent the build-up of harmful sulfatides.

2.3. The development programme/compliance with CHMP guidance/scientific advice

Scientific Advice was obtained from SAWP on 9 occasions between April 2009 and February 2018 on the overall development of Libmeldy and to agree on key elements of the quality, nonclinical and clinical studies to be conducted. The Scientific Advice written reports, as well as minutes of the pre-MAA meeting with EMA

and Rapporteur/Co-Rapporteur meetings conducted in 2019, are provided in Annex 5.14 to the Application form.

Design of the pivotal clinical Study 201222 was first discussed with the Scientific Advice Working Party (SAWP) during a Scientific Advice held in 2009 [EMEA/H/SA/1224/1/2009/PA/PED/ADT/III] in which agreement on the main efficacy and safety endpoints was sought. In particular, the SAWP agreed that the design of this Phase I/II study, i.e. a non-randomised and open-label study, was adequate to address the efficacy and safety of Libmeldy, given the rarity of the disease and the orphan status of Libmeldy. The SAWP also agreed that untreated MLD patients from the SR-TIGET NHx study was an appropriate comparator, provided “these historical controls match the treated population with respect to disease stage, age and genotype”.

In addition, during a Scientific Advice held in 2018 [EMEA/H/SA/1224/1/FU/5/2017/PA/PED/ADT/II] the design of a study in LJ-MLD population to be conducted with Libmeldy was discussed. The SAWP indicated that “a comparison with matched sibling appears to have the least variability” and that “comparison between presymptomatic subjects versus their affected siblings is considered the most informative”. These comments are consequently also applicable to Study 201222 and the Integrated Data Set (Study 201222 and EAPs).

The use of historical controls as comparators was also discussed during the Co-Rapporteur meeting with AIFA during which recommendation was made to clarify that due to ethical considerations, only early symptomatic patients were included in the NHx study; however retrospective data were collected on each individual patient in order to reconstruct as far as possible the dynamics of the disease. A matched-sibling analysis was recommended to be included in the dossier by the assessors along with information on sample size and percentage of missing data at each time point for both treated and untreated subjects.

This advice has been followed by the applicant.

The SAWP agreed with Gross Motor Function Measurement (GMFM) as a primary efficacy endpoint in Study 201222 and recommended to include ARSA activity as a co-primary endpoint. Selected safety endpoints to monitor the safety profile of the treatment procedure were endorsed by the SAWP. Long-term follow-up was also integrated in the protocol, as recommended by the SAWP. Additional discussions occurred with the SAWP on the design of the pivotal study in 2014 [EMEA/H/SA/1224/1/FU/1/2014/ADT/III]. At the time of this Scientific Advice, the proposed sample size consisted of 8 LI and 4 EJ subjects and the plan to submit an MAA supported by an Interim Analysis performed when nine subjects complete their 3-year follow-up visit was discussed. The SAWP considered the proposed sample size to be sufficiently homogeneous and reminded the applicant that “the sample size was more dependent on the condition and not so on statistical requirements.” The SAWP also stated that the proposed interim analysis could be acceptable provided demonstration of a clear outstanding positive benefit/risk profile in this population at 3 years post-treatment. Long-term follow-up of the clinical subjects was also recommended and consequently followed by the applicant. Overall, the applicant’s proposed study design was deemed acceptable for a registrational study supporting a marketing authorisation provided recommendations stated above from the SAWP were implemented in the protocol. The applicant confirms that the protocol was amended accordingly. Of note, the sample size and proportion of pre-symptomatic LI and pre-symptomatic or early symptomatic EJ subjects were revised multiple times during the course of the study with the final design intended to treat a total of 20 subjects.

Extrapolation of the benefit-risk profile of Libmeldy as studied in the LI and EJ population to the LJ MLD variant was discussed in the frame of several Scientific Advice [EMEA/H/SA/1224/1/FU/1/2014/ADT /III; EMEA/H/SA/1224/1/FU/2/2015/PA/PED/ADT/II; EMEA/H/SA/1224/1/FU/5/2017/PA/PED/ADT/II]. The SAWP commented that "Any argument around extrapolation should consider similarity of disease across different

phenotypes and similarity of drug effects across phenotypes (considering the mechanism of action in relation to disease progression). It should also be considered that benefit-risk considerations may differ between populations with different prognosis, progression and severities or disease" [EMEA/H/SA/1224/1/FU/1/2014/ADT/III].

In 2017, during the discussion on the design of a study in LJ patients, the SAWP highlighted that "It is not ruled out that robust positive results from Study 201222 in pre-symptomatic LI and EJ MLD patients and an acceptable safety profile, could support use in pre-symptomatic subjects with more slowly progressive phenotypes which have essentially the same mechanism of disease". Additionally, during the review of the PIP [EMEA-001765-PIP02-15], the PDCO commented that if Libmeldy "proves to be efficacious in children with the most severe forms of MLD, it is likely that its benefit-risk balance is also positive in patients with milder forms of the disease."

The applicant changed the formulation from a fresh formulation to a cryopreserved formulation. With regard to the clinical data generated with this cryopreserved formulation (Study 205756), the SAWP mentioned during the Scientific Advice held in April 2017 [EMEA/H/SA/1224/1/FU/4/2017/PA/PED/ADT/III] that "as there would be data on biomarkers needed for good clinical response from the fresh formulation (e.g. ARSA activity), it would be possible to endorse similarity with the modified cryopreserved formulation based on surrogate variables rather than having to wait for robust clinical outcomes".

Type of Application and aspects on development

The CAT and CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on an unmet medical need in claimed indication, and the expectation that the product would address the unmet medical need in the target population. Considering the severity and irreversibility of the disease, and the fact that this decline can be very rapid in some patients, delayed access will deprive pre-symptomatic and early-symptomatic (LI and EJ) MLD patients of a potentially curative/successful treatment. As such assessment within an accelerated time was recommended.

However, during assessment the CAT and CHMP reverted the evaluation to a standard timetable as the indication, the release testing strategy, and a few other concerns were still under discussion at day 150.

2.4. Quality aspects

2.4.1. Introduction

Libmeldy (also referred to as OTL-200) is an *ex vivo* autologous CD34+ haematopoietic stem cell gene therapy aiming at correcting the genetic defect in metachromatic leukodystrophy patients' own haematopoietic stem and progenitor cells. Administration of Libmeldy is via intravenous infusion. Libmeldy should only be administered once.

The finished product is presented as a cryopreserved dispersion for infusion containing 2-10 x 10^6 of CD34+ enriched cells transduced *ex vivo* using a lentiviral vector (LVV) encoding the human arylsulfatase A (ARSA) gene.

Other ingredients are: 0.9% sodium chloride, 5% dimethylsulfoxide (DMSO) and 7% human serum albumin (HSA).

The finished product is composed of one or more infusion ethylene vinyl acetate bags (50 mL EVA bag). Each infusion bag contains 10 to 20 mL of finished product.

2.4.2. Active Substance

General Information

Libmeldy is a gene therapy medicinal product containing an autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced *ex vivo* using a lentiviral vector encoding the human arylsulfatase A (ARSA) gene. The active substance (AS) is considered as a new active substance in itself. The predicted transgene amino acid sequence has been provided (see section on LVV below).

The section on the active substance is separated into two parts; part 1 for the gene therapy retroviral vector (ARSA LVV) and part 2 for the transduced cells resulting in the active substance (CD34+ transduced cells).

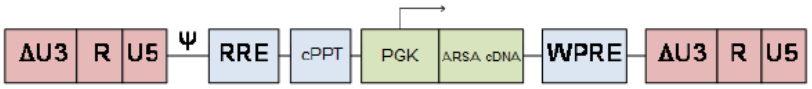
1) ARSA LVV - LENTIVIRAL VECTOR

General information (LVV)

ARSA LVV is a recombinant replication-defective third generation pseudotyped self-inactivating (SIN) HIV-1 - based lentiviral vector that has been modified to carry the human ARSA cDNA sequence. The vector is pseudotyped with the Vesicular Stomatitis Virus envelope glycoprotein G (VSV-G), thus wildtype HIV cannot be generated by recombination among the constructs used to make vectors. The vector is designed to integrate the transgene in the target cells (autologous $CD34^+$ cells) with minimal risk of generating Replication Competent Lentivirus (RCL) and maximising gene transfer efficiency by optimisation of construct design.

The provided general information on the LVV is considered adequate and sufficient.

Figure 2. LVV structure



ΔU3: HIV-1 long terminal repeat (LTR) unique in 3' region (origin: HIV-1)

R: HIV-1 LTR region (origin: HIV-1)

U5: HIV-1 long terminal repeat unique in 5' region (origin: HIV-1)

Ψ: HIV-1 extended encapsidation signal (origin: HIV-1)

RRE: HIV-1 Rev response element (origin: HIV-1)

cPPT: HIV-1 central polypurine tract (origin: HIV-1)

PGK: Human phosphoglycerate kinase promoter (origin: human genome origin)

WPRE: Woodchuck hepatitis virus posttranscriptional regulatory element (origin: Woodchuck hepatitis virus) mutated on nucleotides 1488-1492 at the end of the We1 enhancer (gctga to atcat), to disrupt putative transcriptional element and on nucleotide 1503 (atg to ttg)

Manufacture, process controls and characterisation

Manufacturer (LVV)

The applicant has provided information on the manufacture of the vector. The facilities used in the manufacture of the vector are GMP compliant.

Description of the manufacturing process and process control (LVV)

An appropriate description of the LVV manufacturing process has been provided.

For each step of the proposed commercial manufacturing process a summary, process parameters, and IPCs performed have been provided. Open manipulations of process material are performed in a grade A clean area (biosafety cabinet) by operators trained in aseptic technique using standard operating procedures.

Briefly, the process consists of cell expansion, transient transfection with the transfer vector plasmid (pARSA) and the packaging plasmids (pKG, pKrev, pKLgagpol) in the presence of transfection reagents, followed by incubation, harvest, clarification and pooling of the harvests. Pooled harvest is purified, sterile filtered, filled and frozen. Critical process parameters (CPPs) have been identified and are controlled for each step.

LVV is manufactured in Cell Factories. The harvested material from the Cell Factories is clarified, purified, concentrated and the buffer is exchanged, prior to final fill.

Control of Materials (LVV)

Raw materials (LVV)

An overview of the raw materials used for LVV manufacturing is provided, including information on the manufacturer and specifications.

Starting materials (LVV)

The information provided for plasmid manufacturing and control is generally in line with the expectations. The plasmids are manufactured in accordance with the principles of GMP and testing is in agreement with Ph. Eur. 5.14 requirements. (including identity, genomic integrity, plasmid DNA, host cell DNA, endotoxin and sterility).

The cells used for the LVV manufacturing can be considered standard production cells for various viral vectors, including LVV, used for gene therapy. The manufacture and testing of the MCB is described in sufficient detail. Testing, including adventitious agents testing is generally in agreement with Ph. Eur. 5.2.3. A certificate of analysis has been provided.

Characterisation (LVV)

Characterisation of the LVV includes the provirus and vector proteins and impurities. All genetic (regulatory) elements are identified and the full sequence of the provirus has been provided. Characterisation on PPQ batches is sufficient confirmation that vector with the correct sequence is consistently manufactured.

Vector aggregation was determined by transmission electron microscopy (TEM). Although no aggregates were observed, the applicant evaluated the potential presence of aggregates using multiple methods. It was confirmed that aggregate formation during storage is low.

The applicant presented sufficient data concerning characterisation of a representative batch of ARSA LVV that justify the selection of relevant specifications.

This characterisation included quality attributes such as potency, infectivity, non-infectious viral particles. Potency of ARSA LVV was determined via transduction of reference cell lines, and ARSA transgene activity was evaluated in the same cell lines. The representativeness of the reference cell line was also demonstrated. Correlations between the characterised attributes were demonstrated where appropriate. Impurity data were also evaluated and demonstrated to be reduced to appropriate levels.

Control of critical steps and intermediates (LVV)

For all steps in the LVV manufacturing process several CPPs are identified and controlled. The in-process controls (IPCs) are limited but sufficient to monitor the process and assure process consistency.

Process validation and/or evaluation (LVV)

The LVV manufacturing process has been appropriately validated. Several process performance qualification (PPQ) batches were manufactured. and met the release criteria. Information about the validation of ARSA LVV transportation has also been provided.

Manufacturing process development (LVV)

The applicant has provided a clear overview of the changes made to the vector manufacturing process. The changes are considered sufficiently justified. There are no indications that the changes in manufacturing procedure have negatively impacted product quality or clinical efficacy.

Control of active substance (LVV)

The ARSA LVV release specification includes tests for potency, purity, identity, safety, and general tests e.g. for pH, osmolality. The proposed LVV specifications are acceptable.

Reference standard or materials

An in-house reference preparation is used for the LVV. The protocol for preparation and testing of a new in-house reference standard is considered appropriate.

Container closure (LVV)

ARSA LVV is filled into a sterile, single use cryovial. The information provided on the container closure is adequate and sufficient.

Stability (LVV)

Thirty-six-months long-term stability (at ≤-65°C) data are currently available. Several batches of ARSA LVV are put on long-term stability.

The available stability data support the proposed shelf-life for ARSA LV (36 months) and the proposed storage conditions (≤-65°C).

The provided stability data do not indicate a different stability profile for the vectors from the different manufacturing processes.

Stability studies for several batches of ARSA LVV manufactured according to the commercial process are currently ongoing in accordance with the stability protocol. The studies will be continued until completion.

2) Active substance

Manufacture, process controls and characterisation

Manufacturers

The active substance is manufactured and controlled by Molmed S.p.A in Milan, Italy. MIA and GMP certificate references have been provided.

Description of manufacturing and process controls (AS)

The description of the active substance manufacturing process includes flow charts, narratives, and tabular overviews of the critical process parameters and in process controls.

The process consists of CD34+ cell enrichment, pre-stimulation, transduction, and cell harvest. The description of the manufacturing process is appropriate after additional information was provided upon request.

Information on the batch scale has been provided in the finished product section as this is a continuous batch process. No reprocessing is performed.

Controls of materials (AS)

Raw materials (AS)

An overview of the raw materials used for the active substance manufacturing has been provided, including information on the manufacturer and specifications. Information on the compatibility of the materials used in production with the cells and the potential extractables and leachables has been also provided.

Starting materials

Autologous cells (bone marrow or mobilised peripheral blood)

The information provided on the autologous cells is sufficient. Procurement of bone marrow (BM) or mobilised peripheral blood (mPB) by qualified centres and compliance with relevant directives is confirmed. Virus screening, mycoplasma testing and compliance with EC 2004/23/EC is confirmed by verification of documentation prior to the start of manufacturing. Acceptance criteria for appearance and quantity of the cell suspension were included upon request.

Control of critical steps and intermediates (AS)

In process controls for the active substance manufacturing process are in place. Microbiological control is performed on the starting materials. The finished product is tested for mycoplasma, endotoxin, and sterility.

Process validation and/or evaluation (AS)

Libmeldy active substance is manufactured to finished product as part of a batch process without interruption. Therefore, process validation studies have been provided and can be located in the finished process section.

Manufacturing process development (AS)

Several different active substance/finished product manufacturing processes are identified. Differences include the starting material (BM or mPB), the CD34+ enrichment procedure, the presence or absence of an additional cryopreservation step for the CD34+ enriched cells, the container closure system, and the final formulation

(fresh or cryopreserved). The active substance manufacturing process downstream of the CD34+ enrichment was the same for all clinical batches.

It is noted that apart from these changes there were also the changes in the manufacturing of the LVV vector (as discussed above).

Upon request, the applicant has performed a comparability assessment for clinical batches manufactured at different sites. Some minor differences were observed that could be attributed to the enrichment step or the starting material (BM vs mPB), none of which is expected to impact efficacy or safety. Overall, the data from the clinical batches sufficiently support comparability between manufacturing sites.

Comparability AS manufacturing processes

The main differences in the active substance manufacturing processes are the starting materials and the use of different CD34+ enrichment systems. In addition, changes were made to the vector manufacturing process (see above). The applicant assessed comparability of the clinical batches manufactured from BM and from mPB (see below) and the fresh and frozen formulation (see finished product section) but did not discuss comparability for the several different manufacturing processes. Upon request, this was further addressed, and the applicant performed a retrospective analysis of batches manufactured using vector batches fractionated by the different FP manufacturing processes. The slightly lower cell viability in the cryopreserved finished product is expected and gives no reason for concern.

Overall, comparability of the active substance/finished product manufacturing processes has been sufficiently demonstrated.

Comparability batches manufactured from BM and mPB

Historically, Libmeldy has been manufactured from bone-marrow (BM) derived cells. In order to improve treatment access with an option of a less invasive method than BM harvest, mobilised peripheral blood (mPB) will be added as potential starting material for the commercial manufacturing.

To support the use of either BM or mPB as starting material, the applicant has manufactured several batches from BM and mPB derived from healthy donors and analysed the products before (representative of the fresh formulation) and after cryopreservation (cryopreserved formulation). In addition, batch analysis data from clinical batches manufactured from BM as a fresh (n=29) or cryopreserved formulation (n=4) were compared and an *in vivo* non-clinical study was performed to compare engraftment. The overall approach to assess comparability is in line with previous scientific advice and is endorsed. Data to support the use of healthy donor cells as a surrogate for patient cells are provided and show no differences in cell viability, % CD34+, VCN, clonogenic potential and transduction efficiency.

A significant difference was observed in the %CD34+ cells and clonogenic potential between healthy donor (HD) BM derived batches and HD mPB derived batches, with higher levels in the latter. It is noted that the majority of clinical data is obtained with BM-derived batches, and a higher percentage of CD34+ or clonogenic potential is not expected to have a negative impact on the clinical effect. Some differences in cellular composition are observed when comparing BM and mPB and the total amount of cells was higher for mPB. This difference is not expected to impact efficacy or safety as it is an autologous product and the dose is based on the % CD34+ cells.

The comparison of clinical batches manufactured from BM (n=33) or mPB (n=10) in general confirm the results obtained with healthy donor cells.

Taken together the applicant has sufficiently justified the addition of mPB as a potential source of starting material. It is noted, that analytical results and adverse trends will be further monitored as part of the ongoing process verification programme. In addition, follow-up data will be gathered for the patients treated with mPB derived batches.

Characterisation

Characterisation of the transduced cells includes batch analysis data and extended testing results for all clinical batches. The extended testing includes immunophenotype, clonogenic potential, impurities (residual host cell DNA (discussed in the finished product section) and RCL), and integration site analysis.

The immunophenotype characterisation with respect to the potential cellular impurities and their specific markers is considered sufficient. The relevant cell types have been included.

No replication competent lentivirus (RCL) was detected in the finished product batches. The LOD of the RCL assay was sufficiently justified.

The integration site analysis was performed for all clinical batches and clone abundance is considered appropriate.

The release test for VCN measures the average VCN in all cells. VCN in transduced cells was calculated by the applicant. Upon review, additional information was requested to justify the range of VCN in transduced cells. In response, the applicant provided additional characterisation data, including an analysis of VCN in individual colonies. In addition, the applicant provided further analyses comparing attributes such as distribution of VCN, clinical safety profile and integration site profile for finished product batches across the range of VCNs observed in released finished product batches. The evaluation of the clinical data, in general, gave no indication that the VCN distribution or safety profile of batches is different within the range of VCNs observed. Monitoring of circulating cells (and their VCN) in patients continues to be recommended (see clinical assessment report).

In addition, it is noted that the high percentage of cells with VCN > 10 (up to 44% in finished product batches) could be a reason to be cautious with respect to safety. This is further addressed in the non-clinical assessment.

Specification, analytical procedures, reference standards, batch analysis, and container closure

Specification

All release testing of Libmeldy is performed at the stage of the finished product; therefore, a specification for Libmeldy active substance is not required.

Analytical procedures

No analytical testing is performed on Libmeldy active substance as all release testing is performed at the finished product stage.

Batch analysis

Batch analysis was routinely performed at the active substance stage for Libmeldy fresh formulation when the finished product was stored at ambient temperature; however, when the change was made to cryopreserved formulation and to store the finished product at < -130°C the batch release testing was carried out at the

finished product stage of the manufacturing process. To aid review of data across the manufacturing development history all of the batch analysis data are presented in the finished product section.

Reference standards or materials

Reference standards will not be produced for Libmeldy active substance. Due to the nature of each batch of product being produced from individual patients, it is agreed that it is not possible to produce a suitable reference standard.

Container closure

The active substance and finished product processes are continuous and therefore no information is provided on the active substance container closure. The tubes in which the cells are contained are described in the manufacturing process description.

Stability

Since Libmeldy is produced on an individual patient basis and the active substance is not stored for any longer than it is necessary to ascertain that the quality is sufficient to progress to finished product manufacture, no stability studies are ongoing and none are planned. This is acceptable.

2.4.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Description of the product

Libmeldy dispersion for infusion (FP) is composed of 10 – 20 mL of cryoformulation medium (5% DMSO, 7% HSA, and 0.9% saline solution) containing 2-10 x 10^6 CD34+ enriched cells transduced *ex vivo* using a lentiviral vector encoding the human arylsulfatase A (ARSA) gene per mL.

The product is presented cryopreserved in EVA bag(s). Each infusion bag contains 10 to 20 mL of Libmeldy. The number of EVA bags depends on the total amount of cells and will vary between individual patients. After thawing, the product is administered by intravenous infusion without further manipulation.

Since the total number of cells and concentration of $CD34^+$ cells vary between individual patient batches, the quantitative information regarding strength (total viable cell concentration), volume of dispersion and total number of $CD34^+$ cells per bag and supplied dose of the medicinal product are provided in the Lot Information Sheet. The Lot Information Sheet is included with the cryoshipper used to transport Libmeldy.

Formulation development

Libmeldy was initially formulated as a fresh finished product (i.e. not frozen). In the fresh formulation, the only excipient used was saline. 0.9% w/v Sodium Chloride Infusion is purchased as a medicinal product licensed by a European Union member state. In-house testing performed on the 0.9% w/v Sodium Chloride Infusion has been provided. Endotoxin and sterility are accepted on the supplier's certificate of analysis.

Subsequently, Libmeldy has been formulated to produce a cryopreserved finished product. The development of a cryopreserved formulation is endorsed. Comparability with the fresh formulation is discussed in the section below.

The choice of 5% DMSO as cryoprotectant and 7% human serum albumin as stabiliser gives no reason for concern. The selected target cell concentration of 2-10 x 10^6 CD34+ cells/mL is sufficient to ensure that the minimum required dose can be administered to all patients, irrespective of any limitations to the maximum volume due to the presence of DMSO.

The applicant uses a single source of human serum albumin. It is acknowledged that the albumin used as an excipient in Libmeldy is authorised as a medicinal product in the EU and tested by an OMCL for batch release. The batch release certificate confirms compliance with the Ph. Eur. Sufficient information is available to conclude that the albumin has acceptable quality.

Manufacturing process development

The main changes to the finished product manufacturing process are related to the addition of a cryopreservation step to increase the shelf life. Overall, the provided data support comparability between the fresh and cryopreserved finished product formulations. The container closure system and associated filling method were also changed during development. The changes have been sufficiently discussed and justified. For the commercial process, the applicant intends to use either cells from bone marrow (BM) or mobilised peripheral blood (mPB) as starting material. The majority of clinical batches were manufactured using BM (33 of 37 batches). Data to support comparability between products manufactured from mPB compared to products manufactured from BM have been provided.

To support the change from a fresh formulation to a cryopreserved formulation, the applicant has manufactured several batches from BM and mPB derived from healthy donors and analysed the products before (representative of the fresh formulation) and after cryopreservation (cryopreserved formulation). In addition, batch analysis data from several clinical batches manufactured from a fresh or cryopreserved formulation were compared and, for BM derived batches, an *in vivo* non-clinical study was performed to compare engraftment. The overall approach to assess comparability is endorsed.

Container closure system

The choice of container closure system (CE-marked EVA bag) is considered adequate. Information on microbiological integrity and compatibility is provided in the sections below.

Microbiological attributes

Sufficient information has been provided on the microbiological attributes of the dosage form. Container closure integrity after storage of the EVA bags in the vapor phase of liquid nitrogen was demonstrated.

Compatibility

Sufficient information has been provided on compatibility of the container closure system and extractables/ leachables from direct contact materials (EVA bags, filters, pipettes, centrifuge tube, syringe). The risk to patient safety from extractables and leachables was found to be negligible.

No impact of the cryopreservation/thawing process in EVA bags was observed for finished product batches manufactured from healthy donor cells. In use stability data for finished product batches manufactured from healthy donor mPB or BM support storage up to 2 hours at ambient temperature post-thaw.

Statements are included in the SmPC that the product must not be mixed with other medicinal products, and should not be washed, spun down, and/or resuspended in new media prior to infusion. A shelf life of maximum 2 hours at room temperature (20°C-25°C) once thawed is indicated. This is acceptable.

Manufacture of the product and process controls

Manufacturers

The manufacturers responsible for the manufacturing and release of the finished product are GMP compliant.

Description of the manufacturing process and process controls

The manufacturing process is a batch process that is continuous from receipt of patient material to cryopreservation of the finished product in the final container for use. The finished product manufacturing process consists of cell wash, cell concentration, final formulation, filling and cryopreservation.

After formulation, quality control (QC) samples are collected prior to filling the FP into ethylene vinyl acetate (EVA) cryobags. The primary EVA cryobag is placed in a secondary, labelled, EVA overwrap bag in a Grade B room. This secondary overwrap bag is sealed in a non-classified area. Labelled and packaged finished product and QC samples are cryopreserved at the same time using a controlled-rate freezer. After cryopreservation, each frozen finished product bag is placed into a metal cassette and stored at < -130°C in vapor-phase of liquid nitrogen.

Sufficient detail on the procedures, critical process parameters and in process controls have been provided. The actions taken in case the action limit established for the viability in-process control is not met are appropriately described and justified.

Batch and scale definition

Libmeldy is a patient-specific, autologous product and therefore each batch is manufactured for one patient using autologous patient bone marrow (BM) or autologous mobilised peripheral blood (mPB) as a cellular source from which to derive the starting material. More than one collection may occur in certain circumstances for manufacture of a batch.

The scale of the active substance manufacturing process is dependent on:

(i) the number of mononuclear cells obtained from BM or in the case of mPB, the quantity of CD34+ cells, and (ii) any cell growth during the culture phase.

Sufficient information on the pooling of different starting material collections was provided.

Qualified Treatment Centres (QTC)

The applicant will qualify each of the treatment centres, and they will perform the collection of autologous cells for commercial manufacture of the finished product.

The Product Manual contains detailed instructions for staff responsible for the collection and packaging of autologous cellular source material.

Traceability

Traceability of is carried out in accordance with the qualified treatment centre, manufacturing facility policies, and internal SOPs. The patient code and the finished product batch number are both recorded in batch register, and in the batch record. The finished product and active substance are managed in the same batch record. The system in place assures traceability of the product throughout the entire manufacturing process.

Sufficient information has been provided on traceability, including a flow diagram and brief descriptions of the different components. The finished product label will contain the Chain of identity (COI) ID and batch number assigned to that specific COI ID.

Controls of critical steps and intermediates

An in-process control in the finished product manufacturing process has been identified by the applicant. This is acceptable, as the finished product manufacturing process is very short and only consists of cell wash, concentration, formulation, filling and cryopreservation.

Microbiological control (BactAlert), endotoxin, and mycoplasma are tested at the finished product release, and the results will be available prior to administration to the patient.

Process validation and/or evaluation

As the manufacturing process of Libmeldy is a continuous process (i.e. Libmeldy active substance is manufactured to finished product without interruption), the process validation provided in section P.3.5 of the dossier covers both the AS and FP manufacturing process.

Media fills

Three successful media fill runs were performed at the manufacturing site. The process at maximum manufacturing scale was challenged by e.g. the total volume processed, the number of operations, and the duration of the manufacturing process including finished product filling.

Process performance qualification (PPQ)

The applicant has performed a PPQ study using healthy donor cells from BM and mPB at its manufacturing site. The use of healthy donor cells for PPQ is agreed from an ethical perspective and is supported by comparability data.

The PPQ data demonstrate that the process is capable to consistently produce finished product batches that comply with the specifications.

Impurity removal

The applicant evaluated removal of process related impurities. Results of the evaluation demonstrate that the wash steps are capable of reducing the levels of process related impurities. The applicant provided evidence that the residual infectious virus poses a negligible risk for secondary infection or off-target toxicity.

The applicant provided a statement that no risk of nitrosamine presence in Libmeldy has been identified and the supporting risk evaluation. This is acceptable.

Ongoing process verification

A continuous process verification approach will be used to provide assurance that the process remains in a state of control during routine commercial manufacturing.

A brief summary has been provided of the ongoing process verification that will be implemented. The studies are aimed to monitor process consistency, based on the results of release testing, in process testing and additional characterisation. Results outside the acceptance criteria, and control limit and trend violations will be investigated. Sufficient information has been provided.

Transport validation

The transport validation gives no reason for concern. Both the shipment of the patient cellular source material from the qualified treatment centre (QTC) to the contract manufacturing organisation (CMO) manufacturing sites and the transport the final cryopreserved finished product from the CMOs to the QTC for administration are adequately addressed.

Product specification, analytical procedures, batch analysis

Specifications

The finished product specifications include tests for identity (transgene function (ARSA activity)), identity, potency, purity and safety. Further quality and clinical assessments on whether additional specifications (or modifications) for source material and/or finished product are required will be completed when data from an additional batch becomes available. The applicant is recommended to routinely evaluate the finished product specifications as part of the annual product review. In line with the strategy to set specifications, clinical outcome data review will be part of this process. In addition, as part of the continued process verification process a full evaluation of manufacturing data and tests will be performed, and this will also feed into specifications review. A Major Objection was raised to the applicant's initially proposed release strategy as additional controls would be required to justify the proposal from a quality perspective.

In response to continued review dialogue, the applicant changed to a conventional release strategy, which was acceptable from a quality point of view. The MAH is requested, in the context of an Annex II condition, to reduce the overall time from patient screening to treatment to within the ranges observed during clinical development, while ensuring that product quality remains adequately controlled prior to administration. Reduction of the time needed for product testing and release should be part of these measures.

Analytical procedures

A summary of validated or compendial analytical procedures used for quality control testing of Libmeldy finished product has been provided by the applicant.

In general, clear and sufficiently detailed method descriptions have been provided. Summaries and reports of the method validations have been provided.

Batch analysis

Batch analysis data are provided for several batches manufactured for the clinical studies and for several PPQ batches. The batch analysis results also include tests that are performed for information only.

Justification of specifications

The proposed acceptance criteria are sufficiently justified.

Reference standard or materials

Reference standards will not be produced for Libmeldy finished product. Due to the nature of each batch of product being produced from individual patients it is not possible to produce a suitable reference standard. Given the autologous nature of the product, this is acceptable. Each assay has been developed with its own qualified internal controls and standards.

Container closure system

Libmeldy finished product is filled into one or more sterile, single use, pre labelled 50mL ethylene vinyl acetate (EVA) infusion bag(s) with two available spike ports. Each primary bag is then sealed and packaged into an

overwrap EVA bag which is also sealed, the packaged finished product is then cryopreserved. The frozen packaged configuration is placed in a metal cassette for storage in the vapor phase of liquid nitrogen.

Libmeldy is shipped from the manufacturing facility to the treatment centre storage facility in a cryoshipper, which may contain multiple metal cassettes intended for a single patient. Each metal cassette contains one infusion bag of Libmeldy.

Sufficient information is provided on the container closure system. This includes an overview of the raw material of the different components of the primary container closure system and the manufacturer's specifications of the cryobags. The bags are sterilised. A Certificate of analysis (CoA) and CE-certificate are provided.

The choice of container closure system (CE-marked EVA bag) is considered adequate. Information on microbiological integrity and compatibility gives no reason for concern. The same container is used in the stability study to support the shelf life, however, as a smaller fill volume was used in the stability studies, the bags were heat sealed to reduce the nominal volume and mimic the volume to surface area expected for finished product with the lowest recommended fill volume. This is acceptable.

Stability of the product

Up to 6 months stability data have been provided for finished product batches manufactured from healthy donor material and in use stability data are available at the beginning and end of shelf-life.

It can be accepted that the stability claims are based on data obtained with healthy donor cells, as comparability of healthy donor cells and patient derived cells has been sufficiently demonstrated.

The stability data support the proposed shelf life of 6 months when stored in the vapour phase of liquid nitrogen at < -130°*C* and in use stability of the thawed product for 2 hours at room temperature (20°*C*-25°*C*). All acceptance criteria were met and no trends were observed.

No other stability studies are ongoing and none are planned. Additional stability studies may be performed in support of process changes, process validation, and comparability studies.

Post approval change management protocol(s)

N/A

Adventitious agents

Non-viral adventitious agents

The microbial control is sufficiently described. Appropriate in process controls are in place in the LVV manufacturing process. Microbiological control is performed on the mPB or BM. Upon request a method description and validation were provided. As the manufacturing process is short and includes several wash steps, it is agreed that no further in process tests are in place for microbial control. The final product is tested for mycoplasma, endotoxin, and sterility.

The risk of contamination with animal TSE is appropriately addressed. FBS is the only material from TSE relevant animal species that is used in the manufacturing process. It is derived from countries with negligible BSE risk; relevant certificates have been provided.

Adventitious viruses

An overview of the starting materials, raw materials, and excipients of biological origin used in the LVV, DS, and finished product manufacturing process has been provided. The information provided in the initial application and in response to questions is sufficient and gives no reason for any concern.

The human serum albumin (HSA) used is licensed as a medicinal product in EU. All constituent plasma pools have been tested by an OMCL for virological markers.

In summary, the information provided concerning the adventitious agents safety is adequate and the TSE and virus safety of Libmeldy has been sufficiently demonstrated.

GMO

For discussion and conclusions on the environmental risks related to this GMO product (autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells transduced *ex vivo* using a lentiviral vector encoding the human arylsulfatase A gene) see relevant sections in the CHMP/CAT Assessment Report (see non-clinical section for further information).

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

The provided Module 3 documentation is of reasonable quality. The information on the manufacturing process and its control is sufficient. Valid GMP certificates for gene therapy manufacturing and for QC testing (microbiological, chemical and biological) have been submitted for the relevant manufacturing sites.

Process validation is performed in line with expectations and in general demonstrates that the process is capable to consistently produce finished product batches that comply with the specifications. The proposed specifications are acceptable. Some commitments have been provided in relation to further methods validation. These are acceptable.

Several changes were made to the manufacturing process of the virus vector and the AS/FP. Additional data were provided to demonstrate that the clinical and commercial batches are sufficiently comparable.

The applicant proposes to use either bone marrow (BM) or mobilised peripheral blood (mPB) as starting material. From a patient perspective, the addition of mPB as a potential source of starting material is sufficiently justified. Upon request, additional data were provided that demonstrate comparability. Although some differences are observed in cell composition that can be attributed to the difference in starting material, there are no indications that this results in differences in clinical outcome.

Characterisation of the transduced cells includes batch analysis data and extended testing results for all clinical batches. Upon request the applicant the applicant provided additional characterisation data, including an analysis of VCN in individual colonies. In addition, the applicant also provided further analyses comparing attributes such as distribution of VCN, clinical safety profile and integration site profile for drug product batches across the range of VCNs observed in released drug product batches. The evaluation of the clinical data, in general, gave no indication that the VCN distribution or safety profile of batches varied within the

range of VCN observed. Monitoring of circulating cells (and their VCN) in patients continues to be recommended (see clinical assessment report). The re-evaluation of the finished product specifications as part of the annual product review will also take into account the clinical outcome data.

A Major Objection was raised to the applicant's initially proposed release strategy as additional controls would be required to justify the proposal from a quality perspective. In response to continued review dialogue, the applicant changed to a conventional release strategy, which was acceptable from a quality point of view. The MAH is requested, in the context of an Annex II condition, to reduce the overall time from patient screening to treatment to within the ranges observed during clinical development, while ensuring that product quality remains adequately controlled prior to administration. Reduction of the time needed for product testing and release should be part of these measures.

The provided stability data support the proposed shelf life of 6 months at < -130°C and the claim that once thawed, the shelf life is maximum 2 hours at room temperature (20°C -25°C).

Taken together, the application is acceptable from a quality point of view.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall quality of Libmeldy is considered acceptable when used in accordance with the conditions as defined in the SmPC. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The manufacturing process of the active substance is adequately described, controlled and validated. The active substance is well characterised and appropriate specifications are set. The manufacturing process of the finished product has been satisfactorily described and validated. The quality of the finished product is controlled by adequate test methods and specifications. Adventitious agents safety including TSE have been sufficiently assured.

The CAT has identified the following measures necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product:

The MAH should take measures to reduce the overall time from patient screening to treatment to within the ranges observed during clinical development (median 8.2 weeks; range 6-12.4 weeks). Reduction of the time needed for product testing and release should be part of these measures. (Report on implementation to be submitted 12 months from the Commission Decision, i.e. Dec 2021).

The CHMP endorse the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

The applicant agreed to the Recommendations as identified below.

2.4.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommended several points for further investigation. These included additional tests on starting materials, sourcing and control of raw materials, improvements in analytical methods and re-assessment of acceptance criteria for in-process and release testing attributes for the finished product.

The CHMP endorse the CAT assessment regarding the recommendations for future quality development as described above.

2.5. Non-clinical aspects

2.5.1. Pharmacology

Libmeldy is a cell-based gene therapy medicinal product intended for the treatment of metachromatic leukodystrophy (MLD). The active substance of Libmeldy consists of an autologous $CD34^+$-enriched cell population (containing $CD34^+$ haematopoietic stem and progenitor cells, HSPCs) *ex vivo* transduced with a replication-defective, self-inactivating lentiviral vector encoding the human arylsulfatase A (ARSA) gene under the control of a human phosphoglycerate kinase (PGK) promoter. The active substance is formulated in a cryopreservative solution containing 5% DMSO and 7% HSA (in 0.9% sodium chloride) and is -after thawing- intravenously infused into the patient, who preceding to the treatment underwent busulfan myeloablative conditioning.

For the proof-of-principle studies, the applicant has used murine Lin^- HSPCs as homologue for human $CD34^+$ cells, although the LV vector did contain human PGK and ARSA. This approach is endorsed.

Primary pharmacodynamics

2.5.1.1. Primary pharmacodynamics

In vitro studies

In 2 *in vitro* studies, the applicant determined the transduction capacity of 1) wild-type and ARSA-deficient murine bone marrow-derived Lin^- HPSCs and 2) healthy human $CD34^+$ cord blood-derived HSPCs. These cell populations could be efficiently transduced with GFP-LV or ARSA-LV and transduction of $As2^{-/-}$-derived HSPCs with ARSA-LV resulted (*in vivo*) in supraphysiological ARSA activity. Using the LTC-IC test (to determine early/primitive progenitors, which are the *in vivo* long-term engrafters), considerably more myeloid progenitors than erythroid progenitors were found when ARSA-LV-transduced (human) HSPCs was used as starting material for the LTC-IC test. This is advantageous as the mechanism of action of the product consists of a.o. differentiation of myeloid progenitors into microglia as a crucial step to achieve transgene ARSA expression in the brain. It is not known whether this preference for the myeloid lineage is also observed after engraftment, upon clinical use.

The applicant also conducted an *in vitro* study with healthy human $CD34^+$ bone marrow-derived HSPCs to determine the optimal clinical transduction protocol (i.e. 24h or 48h pre-stimulation, 1 or 2 transduction cycles). Protocol E (i.e. 24h pre-stimulation, 2 transduction cycles) appeared to be superior over the other 2 protocols on bulk level. Nevertheless, a large variation in e.g. VCN, ARSA activity, cell recovery and number of colonies was seen between individual experiments (with all transduction protocols) suggesting that it may not able to produce ARSA-LV-transduced human HSCPs with characteristics within a narrow range. The selected protocol E was subsequently tested in HSPCs from an MLD patient, but some *in vitro* experiments (e.g. regarding yield/$CD34^+$ expression and clonogenic potential of MLD cells) were not conducted. This limits the possibility to conclude about the optimal transduction protocol in cells from patients compared to healthy donors. Nevertheless, protocol E is already used to produce clinical batches. Production of cell batches, their specifications and comparison of transductability between healthy donor and MLD cells is (briefly) discussed in the quality AR.

In vivo studies

Three *in vivo* studies were conducted to determine 1) the engraftment and transgene expression by murine HSPCs, and 2) the treatment efficacy in pre-symptomatic and in symptomatic $As2^{-/-}$ mice.

Engraftment of transduced murine HSPCs was determined in wild type mice. C57BL/6 female mice were IV administered a single dose of $1x10^6$ Lin^- GFP-LV HSPCs per mouse at 6 weeks of age, after a conditioning regimen of total body irradiation (8 Gy). Mice were subsequently followed for 9 months. In addition, an unknown quantity of bone marrow cells of these mice was transplanted into secondary recipients, which were followed for 3 months post-transplantation. The majority of transplanted HSPCs could engraft in both primary and secondary recipient mice, with 53-95% (primary: mean 75%, secondary: mean 69%) of the total blood leukocytes expressing the GFP transgene during the follow-up period. These data show that the GFP-LV was present in stem cells and long-term progenitors.

The therapeutic potential of ARSA-LV-transduced HSPCs was first tested in a pre-symptomatic $As2^{-/-}$ mouse model. Pre-symptomatic female $As2^{-/-}$ mice were IV administered a single dose of $1x10^6$ Lin^- ARSA-LV murine HSPCs (or GFP-LV-transduced cells or untransduced wild type cells as control) per mouse at 6 weeks of age, after a conditioning regimen of total body irradiation (8 Gy). Mice were subsequently followed for 11 months. This study showed that transduced HSPCs and HSPC-derived cells produced supraphysiological levels of ARSA activity in PBMCs. These high levels appeared to be needed for and resulted in normalisation of disease-affected sulfatide metabolism and protection from disease-related neurological abnormalities (i.e. average neurological values similar or even improved compared to values from wild type mice) during the follow-up period.

In a second study to test therapeutic efficacy, symptomatic $As2^{-/-}$ female mice were IV administered a single dose of $1x10^6$ Lin^- ARSA-LV HSPCs (or control cells) per mouse at 6 months of age, after a conditioning regimen of total body irradiation (12 Gy). Mice were subsequently followed for 6 months. This study showed that the majority of transduced HSPCs engrafted and that HSPC-derived cells in blood and liver produced supraphysiological levels of ARSA activity. Based on mean group data, reconstitution of ARSA activity was also observed in the brain, but at lower levels compared to wild type controls. No individual data were available, however, variation in VCN and ARSA activity between mice within a group appeared to be substantial (based on graphical data in the publication).

2.5.1.2. Secondary pharmacodynamics

To assess the safety of ARSA overexpression concerning sulfate and sulfatase homeostasis, the impact of ARSA overexpression on the activity of other sulfatases was evaluated *in vitro* and *in vivo*. The activity of several SUMF1-dependent sulfatases was not impaired by ARSA overexpression. ARSC and ARSE activity was even significantly increased when compared to activity in GFP-transduced HSPCs. This was consistent with the *in vivo* sulfatase activities measured in ARSA transgenic mice, where ARSA activity up to 30-fold compared to controls did not result in haematopoietic or neurological abnormalities. Besides, ARSA overexpression in human HSPCs administered to neonate immunodeficient $Rag2^{-/-}$ γ-$chain^{-/-}$ mice did not impair *in vivo* clonogenic and differentiation potentials of human HSPCs.

2.5.1.3. Safety pharmacology

No dedicated safety pharmacology studies have been performed with human or murine HSPCs transduced with ARSA-LV. Since no overt (histopathological) toxicity in the CNS, cardiovascular or respiratory systems

related to administration of ARSA-LV-transduced HPSCs has been observed in any of the animal studies, the absence of additional studies is endorsed.

2.5.1.4. Pharmacodynamic drug interactions

No dedicated PD drug interaction studies have been performed for Libmeldy, as these were not considered relevant. The absence of these studies can be endorsed.

2.5.2. Pharmacokinetics

2.5.2.1. Methods

The analytical methods for measuring the concentration of cells of formulations administered to animals, engraftment, cell differentiation, vector copies/integrations, and transduction efficiency were generally similar throughout the nonclinical studies and were validated before conducting the GLP studies, which can be agreed.

2.5.2.2. Absorption

It is agreed with the applicant that no dedicated absorption studies were conducted.

2.5.2.3. Distribution

The main premise for using LVV transduced HSCP as a curative treatment for MLD is the repopulation of brain tissue with HSCP-derived transduced microglial cells that can cross correct the enzymatic defect caused by mutated ARSA in the brains. Therefore, studies on the distribution of the genetically modified cells in particular to the brain are regarded as key for the proposed mechanism of action of this product. Distribution of the product was studied in two homologous mouse models (wildtype C57BL/6 and diseased As2-/- mice, Biffi et al. (2004) and Capotondo et al. (2012)) with murine Lin- HSCP transduced with GFP-LV. These homologous models were also used to investigate the effect of various conditioning methods on the redistribution of the genetically modified cells to the CNS (Capotondo et al., 2012). Also, the distribution of ARSA-LV or GFP–LV transduced human (CB) HSPCs was analysed in immunodeficient mouse models (Rag2-/- Il2r gamma chain-/- or NSG mice, Capotondo, 2007). These heterologous models were also used to investigate the effect of the transduction protocol (Report 2017N330774), the difference between fresh and cryopreserved batches (Report 2016N302792) and the particulars of a GMP grade batch (Report 2017N327519; Cesani, 2015). Distribution of the enzyme to the CNS and cross-correction of the disease phenotype in untransduced brain cells through the uptake of ARSA produced by ARSA-LV transduced cells that repopulated the brain, was studied in an FAH-/- animal model in which FAH+/+ hepatocytes stably and selectively expressing ARSA-HA in the liver ARSA-HA LV transduced As2-/- animals. ARSA-HA transgenic animals and untransduced As2-/- animals served as controls (Biffi, 2006).

Distribution of donor murine Lin-HSPCs from CD45.1 C57BL/6 mice transduced with GFP-LV (under control of the PGK promoter) upon injection into recipient CD45.2 C57BL/6 mice was assessed 3, 6 and 9 months post

primary injection/ transplantation and 3 months post-secondary injection / transplantation. Three months post primary transplantation, most of the GFP+ cells appeared to co-express the macrophage markers F4/80 and/or CD11b. In the periphery, GFP signal was observed as expected in *liver* Kupffer cells, *kidney* macrophages, *alveolar* macrophages and cells in red and white pulp of the *spleen*. More importantly, a strong expression of GFP was observed *in the CNS*. In the cerebrum, cerebellum, and the outer cortical layers of the brain near the meninges GFP positive cells with ramified morphology characteristic of microglia were observed. The frequency of these cells in cerebrum and cerebellum increased up to approximately 30% of the resident F4/80+ microglia by 9 months, often located in clusters in various regions of the CNS including the neocortex, hippocampus & fimbria, cerebellar cortex & vermis, and spinal cord. In *the PNS,* GFP+ cells were observed between the somata of sensory neurons in the ganglia and within the endoneurium (i.e. connective tissues around the myelin sheath) of the sciatic nerve, identified as endoneurial macrophages. GFP+ cells in the sensory ganglions and sciatic nerve accounted respectively for 55 to 60% at 6 and for, 77% and 62% of the F4/80+ cells in at 9 months post-transplantation. Three months post-secondary transplantation, GFP+ cells in CNS and PNS were at a frequency similar and with comparable ramified morphology to that observed in the primary recipients, indicating that CNS-migrated cells originated from self-renewing, long-term repopulating HSPCs.

Distribution of donor murine Lin- HSPCs from As2-/- mice, the murine model of MLD, transduced with GFP-LV (under control of the PGK promoter) upon injection into recipient irradiated As2-/- mice was assessed. *Three months post transplantation,* GFP expression was observed in 82% (69-95%) of all blood cell lineages evaluated and *six months post transplantation*, extensive migration of GFP+/F4/80+ cells towards CNS and PNS was observed with particular abundance seen in the white matter-rich areas that are mostly affected by lipid storage, e.g., the corpus callosum and hippocampal fimbria. The majority of GFP+/F4/80+ cells showed a diseased swollen, amoeboid morphology of microglia, and periodic acid-Schiff (PAS) reactivity due to sulfatide storage in the cytoplasm. The abundance of GFP+ cells in the PNS of As2-/- MLD mice was greater compared to C57BL/6 mice, suggesting enhanced recruitment from the BM and/or more rapid turnover of the resident macrophage/microglia population in the nervous system of MLD mice, along with preferential targeting of lipid storage sites by transgene-expressing activated microglia.

Biodistribution of Human CB-derived HSPCs, transduced with laboratory-grade ARSA LV and GFP LV and injected into neonate immunodeficient Rag2$^{-/-}$ γ-chain$^{-/-}$ mice showed efficient engraftment of transduced cells into bone marrow, spleen and thymus that persist up to 20 weeks and differentiate into multi-lineages in haematopoietic organs. The human cells retrieved from the spleen of the transduced mouse show ARSA or GFP expression in the long-term. As both ARSA and GFP LV-transduced cells engraft, persist and differentiate similarly, it was concluded that ARSA (over)expression does not impair *in vivo* clonogenic and differentiation potentials of LV-transduced cord blood-derived human HSPCs when compared to GFP expressing cells.

The effects of three myeloablative conditioning regimens (busulfan (BU), irradiation (IRR) and treosulfan (TREO)) on the reconstitution of various brain cells (macrophage/microglia cells (CD45+CD11b+), parenchymal microglia cells µ-cells, TAµ cells and CNS-Φ cells (meningeal, perivascular, and choroid plexus macrophages) was assessed 5, 14, 45, 90 and 180 days post transplantation in wildtype and As2-/- mice. At Day 5 post-transplantation, GFP+ cells were detected at similar locations and with similar frequency between myeloablative conditions. At Day 14 – 90 post transplantation, (sustained donor chimerism was established in BM) GFP+ microglia cells increased over time for all conditioning regiments but with decreasing efficiency upon BU, IRR and TREO conditioning. The frequency of CNS-Φ cells progressively matched with cells in BM and peripheral blood, suggesting that this population turns over with circulating cells. The frequency of GFP+ TAµ cells was for each time point lower that the CNS-Φ but higher than the µ population and with most GFP+ TAµ cells for BU animals. The frequency of GFP+ µ cells was lower than CNS-Φ cells and increased with time

in the BU and IRR animals, which was not observed in TREO animals up to 90 days post translation. These cells showed the morphology of microglia cells at different maturation states and often appearing as ramified parenchymal cells in clusters, which appeared later and to a lesser extent mature in IRR animals, whereas these cells were lacking in TREO animals.

The myeloablative condition BU and IRR, but not TREO caused a reduction in the frequency of endogenous myeloid cells that was associated with a progressive decline in μ cell relative frequency and with an increase in TAμ cell frequency (FACS). The endogenous microglia in BU treated mice showed de-ramified (senescent) morphology and Annexin V+ staining of the endogenous GFP- cells (apoptosis) within the macrophage/microglia population, which was observed in BU treated and, to a lower frequency, in IRR treated mice. Apoptosis was furthermore confirmed by microarray showing soon after transplant TNF-α mRNA in brains of BU treated and, with a delayed kinetics, in IRR treated animals. These observations indicate that busulfan followed by irradiation is the best method to induce depletion (apoptosis) of endogenous microglia and promoting repopulation of transplanted (and transduced) macrophage/microglia repopulation. Apparently, MLD (As2-/-) microglia showed more sensitivity to the conditioning effect when compared to the wild type (As2+/+) mice.

Biodistribution using pre-GMP ARSA LV preparations generated via three different transduction protocols (A, E and F) were tested *in vitro* and transduced cells from the 'the 2-hit' (E) and 'the 1-hit' (F) protocol were tested in vivo showing engraftment and differentiation in multiple lineages upon transplantation into sub-lethally irradiated Rag2-/-Il2r gamma chain-/- (immunodeficient) mice. There were no statistical differences in *in vivo* repopulation and differentiation capacity between un-transduced / un-manipulated cells and the cells transduced via protocol E and F. However, the number of mice surviving and the number of mice showing engraftment varied for the different conditions (protocol E – Bone Marrow (BM) and Mobilised Peripheral Blood, (MPB) samples and Protocol F, BM and MPB) and were *very minimal* for protocol F-BM. For protocol E-BM, the samples showed a large variation in engraftment, questioning the relevance of statistical analysis and the outcome thereof (see section 3.2.5).

A study (2017N327519) was undertaken to monitor the biodistribution of cord blood derived HSPCs transduced or not transduced with GMP grade lentiviral vector encoding for Human Arylsulfatase A cDNA (ARSA.LV) into neonate Rag2-/-Il2r gamma chain-/- mice after sub-lethal irradiation (IRR). Both untransduced and ARSA-LV transduced CB HPSCs distributed efficiently to and engrafted in bone marrow, spleen, liver, thymus, brain and testis. Cells also differentiated into multiple haematopoietic lineages and this seems similar for the different conditions in the bone marrow, spleen and liver.

The potential for *in vivo* vector mobilisation from human to mouse cells was further investigated using the B2 SINE PCR assay. No signal was detected in DNA samples from the bone marrow (n=7), spleen (n=10) and testis (n=8) of mice transplanted with ARSA LV-transduced human CB-derived CD34+ cells, confirming that ARSA LV distributed stably associated with the human genome and is not transferred into genomes of the transplanted mice cells.

An *in vivo* study was undertaken to compare the engraftment of fresh and cryopreserved ARSA transduced bone marrow CD34+ cells into NSG mice after sub-lethal irradiation conditioning. The engraftment and lineage differentiation of human donor cells in recipient mice and integrated vector copies in bone marrow from NSG mice transplanted with the two formulations were confirmed by FACS and by qPCR for the brain. Overall, these results showed that the engraftment and cell differentiation and VCN in the bone marrow, engraftment in the brain and overall, the frequency of long-term repopulating stem cells was comparable in mice treated with the fresh or cryopreserved formulation.

The reconstitution of ARSA activity in the brain was investigated as part of the study performed in symptomatic MLD mice. In this study, the reconstitution of ARSA activity in the brain by HSPC-derived microglia was also investigated using an FAH -/- animal model in which FAH+/+ hepatocytes stably expressing ARSA-HA were transplanted in liver to provide stable and functional ARSA-HA expression selectively in the liver. Using western blot and immunofluorescence, it was shown that the enzyme distributed to kidney, but also to dorsal root ganglion was at similar levels found in MLD mice transplanted with ARSA-HA LV. However, where ARSA activity was measured in brains of ARSA-HA LV Lin- HSPCs transduced MLD mice, ARSA-HA activity could not be detected in mice in which ARSA-HA was stably and selectively expressed in the liver. This shows that ARSA is not likely to be transduced over the BBB, but that restoring ARSA activity in the brain is likely to be dependent on the reconstitution of µ-cells from ARSA-LV transduced and engrafted HSPCs.

Cross-correction of neurons and glial cells was studied in As2-/- mice (MLD model, symptomatic animals) that were transplanted with ARSA-HA LV Lin-HSPCs. ARSA-HA was detected within lysosomes of HSPC-derived (donor) microglia cells, within endogenous neurons in various areas of the CNS and particularly in Purkinje cells by means confocal microscopy analysis. These data indicate the cross-correction of the phenotype of diseased cells in the CNS and PNS lacking a functional copy of ARSA (-HA) through ARSA-HA produced by transduced microglia cells.

2.5.2.4. Metabolism

It is agreed with the applicant that dedicated studies to address the metabolism of the product are not required due to the biological nature of the product.

2.5.2.5. Excretion

It is agreed with the applicant that a dedicated study to address the risk of release of new virus is not required for the Libmeldy as the potential to replicate is very limited due to the design of the product.

2.5.2.6. Pharmacokinetic drug interaction studies

It is agreed with the applicant that dedicated pharmacokinetic drug interaction studies are not required for Libmeldy.

2.5.2.7. Other Pharmacokinetic drug interaction studies

It is agreed with the applicant that dedicated pharmacokinetic drug interaction studies are not required for Libmeldy.

2.5.3. Toxicology

No single dose and repeat dose toxicity studies were performed. Single dose toxicity was taken along in the long-term toxicity studies. This approach is endorsed.

2.5.3.1. Long-term toxicity

Pre-conditioning regimen

To evaluate the most appropriate pre-conditioning regimen before transplantation in the toxicity studies, (pre-symptomatic) As2$^{-/-}$ mice were IV administered a single dose of 5-7x10^{5} Lin^{-} mock-transduced HSPCs per mouse at 6-8 weeks of age, after a conditioning regimen of total body irradiation ([TBI], 2x 4.5 Gy) or busulfan (4 days 22 mg/kg/day). Mice were observed for 12 weeks after transplantation.

Mortality was present in 1 female mouse in the TBI-treated group, while 9 animals (4M + 5F) died prematurely in the busulfan-treated group. In their report, the applicant mentioned that no deaths before day 28 were present in both pre-conditioning groups, demonstrating the absence of acute toxicity due to the regimens and/or transplantation. In the GLP long-term toxicity study, however, mortality in some animals only occurred in the first 28 days post-transplantation, while the same TBI protocol (i.e. 2x 4.5 Gy) was used. Nevertheless, this mortality (7 of the 109 animals) was considerably less than with busulfan in the pre-regimen testing study. In the PK study on myeloablation for microglia reconstitution, the busulfan dose was comparable to the current study. However, no details about busulfan-related mortality were provided.

Engraftment in peripheral blood (5 weeks post-transplantation) and bone marrow (termination, i.e. premature death or week 12) was comparable between both pre-conditioning regimens. Because TBI was better tolerated, the applicant used this regimen for pre-conditioning in the long-term toxicity studies.

Clinically, busulfan instead of irradiation is used as a pre-conditioning regimen. Busulfan is less toxic in humans and (based on animal data) is expected to result in a more pronounced depletion and replacement of microglia compared to irradiation (see Pharmacokinetic section).

Toxic and tumorigenic potential

To evaluate the toxic and tumorigenic potential of ARSA-LV-transduced HSPCs, two non-GLP studies were conducted, in which wild type or As2$^{-/-}$ mice were IV administered a single dose of 1x10^{6} Lin^{-} ARSA-LV HSPCs (or control cells) per mouse at 1-2 months or 7-9 days of age (young adults and neonates, respectively), after a conditioning regimen of total body irradiation (11 Gy adults, 7.5-8.5 Gy neonates). Mice were followed for 8-12 months after transplantation. In both studies, mortality was primarily observed within the first 29 days after transplantation, which was considered related to the pre-conditioning regimen of total body irradiation.

In the first study (Report 2018N364205) in adult As2$^{-/-}$ mice, hepatocellular lipid vacuolation, necrosis and neoplasms were found, with the highest frequency and severity in the ARSA-LV HSPC-treated group (i.e. 5 of 25 mice with neoplasms). To further investigate this, the second study (Report 2018N364207) was conducted in wild type and As2$^{-/-}$ mice of two age groups. Hepatocellular changes (such as steatosis and inflammation) were observed in all groups, although neoplasms were only found in As2$^{-/-}$ neonates (i.e. in the different test groups). Although there did not appear to be a relation between ARSA-LV HSPC treatment and tumorigenicity, the applicant conducted a similar study under GLP conditions. As2$^{-/-}$ mice were IV administered a single dose of 1x10^{6} Lin^{-} ARSA-LV HSPCs (using the clinical ARSA-LV) or Lin^{-} mock-LV HSPCs per mouse at 6-8 weeks of age, after a conditioning regimen of total body irradiation (2x 4.5 Gy). During the observation period of 12 months after transplantation, only 2 hepatocellular neoplasms were found (1 in ARSA-LV group, 1 in mock-LV group). This lower number of neoplasms (compared to the non-GLP studies) could be due to differences in the pre-conditioning regimen and/or genetic background. Considerable clinical and pathological effects present in all irradiated and HPSC-transplanted groups (mortality, decreased body weight gain and food consumption, decreased mean body weight at termination with an increase in relative

organ weights, impact on erythrocyte-related values, irradiation-related injury to a.o. kidneys, thymus, eyes, gonads, Harderian gland, and adrenal glands) were considered related to the pre-conditioning and transplantation regimen.

2.5.3.2. Genotoxicity

The genotoxic potential of Libmeldy was evaluated via analysis of vector integration sites, *in vitro* immortalization, and *in vivo* oncogenicity.

To assess common integration sites (CIS) of the Libmeldy-related LV, healthy human $CD34^+$ ARSA-LV HSPCs derived from bone marrow, peripheral blood or cord blood were IV administered to 3-day old $Rag2^{-/-}$ $IL2rg^{-/-}$ mice, after a conditioning regimen of total body irradiation (4.5 Gy). Mice were subsequently followed for 10-12 weeks. *In vitro* as well as *in vivo*, CIS were found for this LV, although no enrichment for a specific gene class was observed. These CIS were present in specific genomic regions (i.e. megabase-wide) and did not target a single gene (i.e. kilobase-wide) as is the case for oncogenic CIS.

Determination of the LV integration profile was also conducted in $As2^{-/-}$ mice transplanted with Lin^- ARSA-LV HSPCs (as part of the GLP long-term toxicity study). In these mice, polyclonal reconstitution was found, although clonal diversity was lower than observed *in vitro* (as expected, because not all clones will engraft). The LV integrated within transcription units (thereby targeting genes such Sfi1) without preference for promoter or regulatory elements. No preferential expansion of integration near proto-oncogenes or tumour-suppressor genes was observed.

To assess the insertional transformation of the Libmeldy-related LV, wild type murine bone marrow-derived Lin^- HSPCs were transduced with GFP-LV and cultured in an *in vitro* immortalisation assay. Results indicated that the LV does not pose a considerable risk for insertional mutagenesis with transformation and sustained growth of HSPCs *in vitro*.

Finally, the oncogenicity of the Libmeldy-related LV was assessed with GFP-LV Lin^- HSPCs from $Cdkn2a^{-/-}$ mice (i.e. FVB mice susceptible to cancer-triggering genetic lesions), which were IV administered to wild type FVB mice (6 weeks of age, after total body irradiation with 2x 5.75 Gy, $7.5x10^5$ cells/mouse). In this model, the LV appeared to have a preference to integrate within transcription units, but not specifically close to promoters (in contrast to retroviruses). GFP-LV transduced cells developed into tumours, but there was no tumour acceleration compared to animals treated with untransduced cells. No overrepresentation of any gene ontology category in pre-transplanted cells neither a selection for a specific gene class in the tumour cells was observed. Thus, despite high numbers of integrations and integration of the PGK promoter in transcription units, Libmeldy-related LV showed no considerable insertional transformation potential in the tumour-prone mice, which is most likely due to the absence of active LTRs (i.e. LV is self-inactivating).

2.5.3.3. Reproductive and developmental toxicity

No dedicated reproductive and developmental studies are undertaken. As the cells are transduced *ex vivo* with a replication-incompetent lentiviral vector, the risk for transduction of the gametes and consequently the risk from germline transduction can be regarded as very limited and rather theoretical. The applicant addressed the risk of secondary transduction, which appears very limited upon the use of clinical-grade viral vector and the presence of human serum. Bystander cell transduction, or the potential for vector mobilisation, was not observed to occur *in vivo* in animals, suggesting that the risk of transduction of gametes via vector mobilisation and secondary transduction is also very low or absent.

2.5.3.4. Other toxicity studies

Detection of replication-competent LV (RCL) is assessed in the quality AR. The presence of residual RCL did not raise specific non-clinical safety concerns.

To assess the potential carry-over of infectious vector particles to off-target cells, Libmeldy-related LV shedding was investigated *in vitro* and *in vivo*. Healthy human $CD34^+$ HSPCs isolated from cord blood were transduced with laboratory-grade and clinical-grade LV, using the clinical transduction protocol. Transduced HSPCs cultured with a cell line or primary MSCs showed that vector carry-over from HPSCs to secondary targets was possible, but that the use of a clinical-grade vector and the addition of human serum, proteinases or heparin could considerably reduce or almost completely inhibit this secondary transduction. Bystander cell transduction was also absent *in vivo* ($Rag2^{-/-}$ γ-$chain^{-/-}$ mice transduced with human HPSCs), as no human genome was found in murine cells. Moreover, Cesani et al. (2015) mentioned that 3 MLD patients with high-level engraftment of transduced cells did not have LV DNA in their bone marrow-derived MSCs above the detection threshold (1-1.5 year after transplantation). These findings suggest that the risk of LV vector shedding is considered low.

No dedicated non-clinical studies to evaluate local tolerance, antigenicity and immunotoxicity, dependence, metabolites and impurities were conducted. The absence of these studies was sufficiently justified and can be endorsed. No additional studies are warranted.

2.5.4. Ecotoxicity/environmental risk assessment

General

It is the responsibility of the applicant that the molecular characterization of the plasmids used for the production of the ARSA LVV, and/or the ARSA LVV used for transduction of the CD34+ HSPCs are complete and correct. This is the starting point for the environmental risk assessment.

Concerning the CD34+ HSPCs, the Good Practice on the assessment of GMO-related aspects in the context of clinical trials with human cells genetically modified using retro/lentiviral vectors (version 3) is applicable.

This implies that where the applicant demonstrates that:

(i) there is no or negligible risk of formation of replication-competent virus

and

(ii) the finished product is free of residual infectious viral vector particles that are capable of being released

the overall risks for human health (individuals other than the patient) are then considered negligible.

The applicant has sufficiently demonstrated there is no or negligible risk for the formation of RCL.

Concerning the residual LVV ARSA particles, based on theoretical calculations the applicant states that negligible amount of the residual infectious LVV is present in the drug product. However, there are shortcomings in the information provided by the applicant (the maximum level of viral vector input was not sufficiently substantiated, and the formula was not used correctly) and in the experimental data submitted on the residual LVV in the product. It is thus anticipated that here will be a considerable amount of residual viral

particles present in the drug product, contrary to the assumptions of the applicant. However, based on what is known from LVV (e.g. with regards to species specificity and stability/inactivation etc), the risk of residual infectious LVV in a drug product can, in general, be considered limited. It is also noted that in the treated patients, no risk due to residual LVV has been identified while these patients were significantly immune-compromised at the time of administration. Given these considerations, risks for human health (individuals other than the patient) of the residual LVV in this product are not expected.

Therefore it can be agreed that the risk to the environment of the residual infectious particles in the product can be considered negligible.

2.5.5. Discussion on non-clinical aspects

Relevance of the As2$^{-/-}$ mouse model

The applicant has used the As2$^{-/-}$ mouse as a disease model for MLD in children. The applicant stated that neuropathological changes and symptoms observed in these mice are comparable to those patients, although symptoms are milder and disease is not life-threatening (i.e. normal life span). Indeed, while the overall pattern of sulfatide storage was comparable between mice and men, Ramakrishnan et al. (2007) showed that the amount of sulfatide accumulation in As2$^{-/-}$ mice is not sufficient to mimic toxic levels related to neuronal damage in humans, and that overexpression of a sulfatide-synthesising enzyme is needed to produce widespread demyelination which is observed in the clinic. As a consequence, the ARSA-deficient mouse model does not resemble the devastating neurological deficits found in (advanced) human disease (Hess et al. (1996). Because of the milder phenotype of the As2$^{-/-}$ mice model, the effect of the ARSA-LV HPSC treatment on neuronal damage could be overestimated when this is directly extrapolated to MLD patients. In addition, the variability in MLD disease, in age of onset (late infantile, early juvenile, late juvenile), and the underlying ARSA gene mutation are not mimicked in the animal model (i.e. complete absence of ARSA activity, see Hess et al., 1996). The mouse model appears to only reflect an early stage of late infantile MLD.

Although there are some limitations of the As2$^{-/-}$ mouse model, the applicant believed that the *in vivo* PD studies have provided valuable data on the potential of ARSA LV-transduced HSPCs to engraft and reconstitute ARSA activity in these mice. This value is recognised and it is considered that the *in vivo* PD data provide a rationale for clinical treatment. However, further extrapolation of non-clinical data to the clinic is not possible because of the limitations of the mouse model relevance for different MLD patients and differences in a.o. cell dose, LV/cell batches, pre-conditioning regimen and manufacturing process.

Notably, when compared to *in vivo* VCN values found in the PBMCs of treated MLD patients, VCN values in treated symptomatic animals were considerably higher, which (again) may lead to an overestimation of the treatment effect in terms of prevention of onset or reversibility of disease.

In conclusion, while the used animal model may be able to provide proof of principle, more quantitative extrapolation of treatment effects (e.g. effective dose, thresholds for VCN levels or ARSA activity needed for disease correction, duration and course of ARSA expression) is not possible based on this animal model and discriminative effect for the various MLD variants at different stages of disease (i.e. early and late events) is very limited. This is further discussed below.

In vivo PD studies

From PD experiments, a relationship between VCN and ARSA activity as well as clinical scoring data is unclear. The applicant was asked to elaborate on a potential relationship using raw data. These raw data appeared not to be available and only part of the relative values for the treated animals (compared to wild type values) could be presented, which also did not point towards a clear correlation between ARSA activity, VCN, clinical scoring and histopathology. It has to be noted that submitted non-clinical PD data are mainly composed of the original testing facility (SR-TIGET) studies and bibliographic literature substituting and/or supporting certain test(s) or studies. Nevertheless, the applicant was of the opinion that a relationship between VCN and ARSA expression existed, but was less visible in mice due to decreased transcriptional activity from the human PGK promotor in mice as compared to human. This is not a very plausible explanation, as a lower transgene expression and ARSA activity could still correlate with VCN. From the data it only appeared that higher VCN and ARSA activity results in better clinical performance of the animals.

The observed difference in VCN and ARSA activity between group A (i.e. lower mean VCN and ARSA activity) and B (i.e. higher mean VCN and ARSA activity) in *in vivo* mice experiments could have been caused by batch variation and related differences in a) infectivity of the batch, b) transduction efficiency of the cells, c) irradiation dose, causing variability in level of myeloablation, or in d) condition of the transduced cells. All of these conditions might have contributed to the observed differences, but absence of raw data leaves a definitive explanation / conclusion open.

In vivo ARSA activity in PBMCs of animals treated pre-symptomatically was considerably higher (mean about 7-fold) compared to symptomatic animals (mean group A = 2.8-fold, mean group B = 4.2-fold), using a comparable transduction protocol. The applicant noted that this interstudy ARSA activity variability might be explained by a better (or more consistent) reconstitution of ARSA activity in pre-symptomatic animals , which could be related to differences in the protocols of the pre-symptomatic and symptomatic studies, such as age/size of the animals at transplantation and sampling, the conditioning regimen, the transduction protocol, and/or size of control group(s). It is very likely that (some of) these factors have added to the differences in mean ARSA activity between pre-symptomatic and symptomatic animals.

According to the applicant, a minimal ARSA activity level of 4-fold the wild type level in PBMCs is required for efficacy (i.e. prevention of further disease progression) in disease-affected mice. It was, however, not clear how the applicant had determined this threshold value. In addition, the minimal VCN and ARSA activity needed in the other HSPC-derived cell types (e.g. Kupffer cells, CNS cells) for *in vivo* improvement, and whether or not this minimal value could be derived from the PBMC threshold value, was not described. Whether or not a minimal ARSA activity in clinical studies is also needed was not known. The applicant was therefore asked to elaborate on the relation between ARSA activity and *in vivo* performance of diseased animals versus patients, by explaining 1) the rationale for the proposed non-clinical threshold of ARSA activity for *in vivo* efficacy (in MLD mice ≥ 4 fold in PBMCs compared to wild type levels), 2) how this threshold relates to minimal ARSA activity in other cell types, 3) whether there is a minimal and optimal ARSA activity level needed in patients and a relation thereof to the proposed non-clinical threshold. The threshold proposed by the applicant appeared to be based on the mean ARSA activity value in group B (> 4-fold), where better *in vivo* treatment effects were found compared to group A (mean ARSA activity < 4-fold). Together with the data from pre-symptomatic mice (ARSA activity ≥ 4-fold), this would support the choice of the threshold value. Although the mean ARSA activity value in group B was higher than in group A, there were several animals in group A with ARSA activity > 4-fold and animals in group B with ARSA activity < 4-fold:

(i) Group A ≥ 4-fold = 4 of the 18 animals for which individual data were available

(ii) Group B with < 4-fold = 9 of the 23 animals for which individual data were available

However, individual data did not discriminate between a group ≥ 4-fold and < 4-fold ARSA activity with regard to neurophysiological and pathological performance. Thus, a threshold of ≥ 4-fold ARSA activity in PBMCs seems not be justified based on the individual animal PD data. Moreover, an estimation for a comparable threshold in brain or liver is not valuable either. The applicant admitted that a proposed non-clinical threshold could not be directly related to a minimal/optimal ARSA activity in patients. Instead, patient genotypic differences and disease status at time-of-treatment are more likely to impact the clinical outcome than PBMC-related ARSA activity, which is endorsed.

Whether there is also a minimal ARSA activity level needed in pre-symptomatic animals was not described. Taking the individual variation in neurological functioning and pathology into account, the applicant was asked to 1) provide individual animal data on *in vivo* VCN, ARSA activity and neurological scoring (functions and pathology), 2) elaborate on a (potential) ARSA activity threshold for *in vivo* efficacy in pre-symptomatic $As2^{-/-}$ mice, and 3) discuss the relation of this threshold to minimal and optimal ARSA activity levels in pre-symptomatic MLD patients.

No individual animal data was available. Based on the data from Biffi *et al.* (2004), the individual ARSA activity in PBMCs of treated pre-symptomatic animals was 4- to 8-fold at 7 months post-transplant. Therefore, the applicant concludes that the ARSA activity threshold in the PBMCs of these animals was ≥ 4-fold the wild type levels. However, such a threshold could only be supported by data from treated animals with ARSA activity < 4-fold that would result in a considerably lower neurological scoring compared to animals with ARSA activity ≥ 4-fold. As these data were not available and considering the individual variability in neurophysiological performance in the different groups, a threshold for pre-symptomatic animals could not be proposed. Considering the difference in MLD classification of patients compared to animals and the absence of a data-supported ARSA activity threshold in pre-symptomatic animals, it was also not possible to elaborate on a potential quantitative relationship between ARSA activity levels in animals and patients, as acknowledged by the applicant.

Overall, the available non-clinical pharmacodynamics data show that ARSA LV can transduce murine Lin^- and human $CD34^+$ HSPCs, resulting in integration of ARSA LV into the DNA and expression of the ARSA protein from it. After pre-conditioning of the mice using irradiation or via busulfan treatment, these cells are able to engraft and persist in $As2^{-/-}$ mice (lacking the ARSA gene in their bone marrow-derived cells) and in wild type mice, and express the ARSA protein locally (including the CNS) and systemically. The resulting functional ARSA protein is able to alleviate disease symptoms as exhibited in symptomatic As2-/- mice and protection from development of disease symptoms in pre-symptomatic As2-/- mice. Together, these data provide a rationale for the treatment of MLD patients with ARSA LV-transduced autologous $CD34^+$ HSPCs after pre-conditioning of the patients with busulfan.

Pharmacokinetics / biodistribution

The pharmacokinetic studies with either mouse equivalent cells in wild type or diseased mice or with human cells in immune-compromised animals show that genetically modified ($CD34^+$) HSPCs distribute and repopulate also to the brain. Data suggest that this occurs most efficiently upon busulfan conditioning, followed by total body irradiation. Treosulfan preconditioning seems to result in less effective repopulation of the microglia cells in the brains as treosulfan is unable to cross the BBB. As a consequence, treosulfan does not remove the endogenous microglia cells and thereby does not provide an empty niche for the transplanted HSPCs to repopulate the brains with microglia cells.

In vivo biodistribution studies in mice indicated that busulfan is the most favourable method to induce depletion of endogenous microglia and promote macrophage/microglia replacement with transduced cells. Apparently, MLD ($As2^{-/-}$) microglia showed more sensitivity to the conditioning effect when compared to the cells from wild type ($As2^{+/+}$) mice. In toxicology studies using busulfan, it appears that animals have died from this myeloablative condition. This was not discussed in the publication of Capotondo (2012) in PNAS. The applicant was asked to submit information on the effect of busulfan on animal survival and the consequence on the interpretation and reliability of the Capotondo study (2012) in PNAS concerning the preference for conditioning regimen.

The applicant explained that a dose of 60-100 mg/kg was usually well tolerated by the animals. While not providing full myeloablation, this dose produced high degrees of stable chimerism, sufficient to provide engraftment of 80% of GFP positive cells. Generally, there was nothing noted about mortality in the raw data in the pilot study (Report 2015N232105) conducted for selection and optimizing the pre-conditioning regimen, also not in the Capotondo study, indicating that the observed mortality was not regarded as an remarkable issue. Based on the applicants response it seems likely that the used busulfan regimen was not detrimental to the animals. Furthermore, the applicant provided more support for the use of busulfan as preconditioning regimen to obtain more efficient brain engraftment of the transduced cells, which may (partially) be due to the difference in the inflammatory response in the brains, which is less severe in the case of busulfan treatment as compared to irradiation.

Distribution of the ARSA enzyme to the brain was also studied in an $FAH^{-/-}$ animal model in which $FAH^{+/+}$ hepatocytes stably expressing ARSA-HA were transplanted in the liver to provide stable and functional ARSA-HA expression selectively in the liver. ARSA-HA transgenic animals and ARSA-HA LV transduced $As2^{-/-}$ animals served as controls. This study indicated that ARSA enzyme is not likely to be transduced over the BBB, but that restoring ARSA activity in the brain is rather dependent on reconstitution of µ-cells from ARSA-LV transduced-and-engrafted HSPCs. In the same study, cross-correction of the disease phenotype in neurons and glial cells of $As2^{-/-}$ mice transplanted with ARSA-HA LV Lin-HSPCs was observed. ARSA-HA was detected within lysosomes of HSPC-derived microglia cells, but also within neurons in various areas of the CNS and particularly in Purkinje cells, indicating that cross-correction of the phenotype of diseased cells in the CNS and PNS could be restored by secreted ARSA-HA produced by transduced microglia cells.

Biodistribution using pre-GMP ARSA LV preparations generated via three different transduction protocols (A, E and F) was tested *in vitro* (Report 2017N330774), and protocol E showed the highest efficiency. Transduced cells from two protocols (i.e. E 'the 2-hit' and F 'the 1-hit') were used to investigate stable engraftment and differentiation in multiple lineages upon transplantation into sub-lethally irradiated *Rag2*$^{-/-}$ *Il2r gamma chain*$^{-/-}$ (immunodeficient) mice. There were no statistical differences in *in vivo* repopulation and differentiation capacity between un-transduced / unmanipulated cells and the cells transduced via protocol E and F. However the limited number of samples made the relevance of the statistical analysis questionable together with a lack of explanation on the seemingly preferential thymic engraftment compared to bone marrow and spleen, and unclear differences in LV integration in the *in vivo* part of this study may not yield firm conclusions on the most effective transduction protocol. Support for pursuing protocol E to the clinic comes solely from *in vitro* data (see section 3.2.1).

Biodistribution of human CB derived HSPCs transduced or not transduced with GMP grade ARSA.LV into neonate *Rag2*$^{-/-}$ *Il2r gamma chain*$^{-/-}$ mice after sub-lethal irradiation (IRR) (study 2017N327519) resulted in efficient engraftment in bone marrow, spleen, liver, thymus, brain, and testis. Distribution to the brains was only presented for 3 animals. Furthermore, data seemed to indicate that cellular differentiation was similar

for the various tested conditions (unmanipulated (UM), mock transduced (UT) and ARSA LV transduced) in the bone marrow, spleen, and liver. However, transduction, either with ARSA LV or UT seemed to influence the engraftment, preferring thymus for bone marrow, and it appeared that UT and ARSA LV transduced cells differed in their thymic CD4/CD8 ratio (i.e. UT more thymic $CD8^+$ T cells and ARSA LV having more thymic $CD4^+$ T cells). An explanation on the preference of engraftment to the thymus over the bone marrow and the difference in thymic CD4/CD8 ratios for the different conditions was requested. The applicant clarified that experimental set-up (different sessions of transplantations among which the groups are not equally spread) and the limited number of animals in the UM group may have contributed to the inter-group variability observed in the study. Exempting these animals as outlier results from the statistical calculation showed that there was no real difference between the groups concerning the CD4/CD8 ratio. This explanation and this approach can be accepted and also exemplifies the importance of the design of the experimental set-up.

Furthermore, it should be noted that in a biodistribution study in the the applicant's programme, also a CD4/CD8 ratio ≥1 was observed in young *Rag2*$^{-/-}$ *IL2r-gamma chain*$^{-/-}$ mice. This was also observed in literature by Traggiai et al. (2004), who reported in their paper that the thymus contained CD4 and CD8 single positive T cells in 1:1 to 4:1 ratios. Thus, next to the above explanation on different sessions of transplantation, the thymic CD4/CD8 ratios appear also to be strain-specific. No effect on the thymic $CD4^+$ and $CD8^+$ subpopulations of MLD patient treated with Libmeldy is expected. Indeed, clinical data obtained so far show sufficient thymus engraftment and $CD4^+$ and $CD8^+$ T cell numbers in peripheral blood. Vector mobilisation from human to mouse cells was not observed in mice transplanted with ARSA LV-transduced human CB-derived $CD34^+$ cells, suggesting a low risk of mobilization and transduction of bystander cells, including germ cells.

Toxicology studies

Long-term toxicity

Intravenous treatment of As2$^{-/-}$ mice with a single dose of $1x10^6$ ARSA-LV-transduced Lin^- HSPCs resulted in engraftment and persistence of these cells without considerable product- or ARSA overexpression-related toxicity or tumorigenicity. Clinical and (histo)pathological abnormalities found in the mice were related to the disease, to aging, and to the pre-conditioning/transplantation regimen. However, although the dose used in these studies was higher than currently proposed for humans (which is acceptable for toxicity studies), differences in transduction protocol compared to the clinical manufacturing process exist. Therefore these studies may be of limited relevance for the clinic. Nevertheless, from these studies can be concluded that there appear to be no safety issues, other than issues that are to be expected with 'normal' HSPC transplantation (including pre-conditioning).

In the non-GLP toxicity studies, ARSA activity appeared to be considerably lower *in vivo* compared to *in vitro* levels. In some animals, even no ARSA activity could be detected despite positive VCN values. Moreover, both *in vitro* and *in vivo* VCN values were high, but variable. Most important, there did not appear to be a positive relation between *in vivo* terminal VCN and ARSA activity. According to the applicant, this could be the result of *in vivo* gene silencing (Capotondo et al., 2007, ARSA transgenic mice). However, this hypothesis was not further investigated and the clinical relevance of this potential silencing was not discussed. The applicant was asked to substantiate their explanation related to the absence of VCN-ARSA activity correlation with (literature) data and provide a discussion on the potential mechanism of silencing, including whether this occurs in HSCTs and/or (specific lineages of) differentiated cells. Clinically, a more positive VCN-ARSA activity correlation appeared to be present. The applicant was asked to elaborate on the potential of occurring of LV cassette silencing in patients and the possibility to monitor and/or steer such events. The applicant provided a detailed literature-based overview of the occurrence and potential mechanisms of gene silencing, resulting

in a lack of/reduced transgene expression in (mainly) undifferentiated cells from both animals and human. The applicant considered the potential gene silencing in ARSA transgenic mice, which most likely occurred due to very high VCN values (in PBMCs), not clinically relevant because VCN in the DP (and in the patients) has always been below 10. Nevertheless, these were only mean VCN values and individual cells could have considerably higher VCN values (see below, genotoxicity subsection).

To further support their statement that gene silencing is not clinically relevant, the applicant stated that ARSA activity has been stable over time in patients without signs of decline. However, in the CSF of several late infantile and pre-symptomatic early juvenile patients, ARSA activity showed a decline. Nevertheless, whether gene silencing would result in a comparable decline or a more extensive decrease in ARSA activity is not clear. As such, no conclusion related to the level of ARSA activity and potential gene silencing in MLD patients can be drawn.

Considering that LV-related gene silencing has been observed in human cells, there will be a potential that in patients infused with ARSA-LV $CD34^+$ HSPCs gene silencing will occur. However, the applicant stated that ARSA activity, which is considered an important clinical efficacy endpoint, is monitored closely in treated MLD patients. Although potential gene silencing will not be further investigated and it is not clear whether steering of such silencing would be possible in humans, close monitoring of both ARSA activity and clinical scoring of patients is considered sufficient to detect potential loss of efficacy.

Risk for mutagenicity

Lentiviral vectors integrate in the host DNA. When insertion occurs in pro-oncogenes or genes protecting cells from proliferation, this may have the potential to result in oncogenic transformation. To address this theoretical risk the applicant provided an analysis on insertion site which suggest that -related LV is not expected to selectively integrate in narrow genomic regions related to oncogenesis nor close to promoter or regulatory elements and would not result in the transformation of cells, thus the mutagenic risk will be sufficiently low as LV vectors are not considered to insert in genes related to oncogenicity. The presented data included an integration site analysis presented in two reports (Biffi *et al.*, 2011 and Report 2016N302788) and from the first 7 patients treated with ARSA-LV-transduced HSPCs (Biffi *et al.*, 2013 and Sessa *et al.*, 2016). The non-clinical data showed that on the individual gene level, there was no significant change in frequency between *in vitro* and *in vivo* datasets ($CD34^+$ HSPCs), thus no enrichment in preferentially targeted genes when the product is used *in vivo*. Within these datasets, 31 common integration sites (CISs) were identified, although the individual CISs targeted differed considerably between the datasets. Nevertheless, the data from a.o. Biffi et al. (2011) showed that integration patterns with the LV corresponded largely to those observed for other LVs (i.e. within transcription units and not in promoter/regulatory elements). Also, LV CISs did occur in wide regions of high-integration density and not as a cluster within a small region (as would occur in case of genotoxic CISs).

Integration site analyses of patient samples (bone marrow and peripheral blood) from different time points post-transplantation confirmed the non-clinical data: LV integration occurred preferentially within transcriptional units and there was no outgrowth of specific clones (polyclonal reconstitution), which was especially apparent after long-term follow-up. Moreover, the integration patterns between individual patients and with patient data from another LV-transduced cell product were comparable (i.e. same gene classes preferentially targeted). These CISs and other overrepresented gene classes are not considered to be related to expression of oncogenes or genes associated with cell proliferation.

Nevertheless, integration site analyses data described by the applicant were predominantly derived from the first 7 MLD patients, which have been treated with batches with VCN values ranging between 1.7 and 4.4 copies. At 1-year post-infusion, the mean VCN values in PBMCs of these patients ranged from 0.1 to 1.4 copies. However, some individual cells have VCN values > 10, which could give rise to a safety concern. Moreover, since there appears to be an increase in the percentage of individual cells (colonies) with VCN values < 2 in patients compared to the DP, there could be a concern related to sustained efficacy. The individual data clarified that most patients had been treated with batches containing a considerable amount of individual cells with VCN < 2 or VCN > 10, although the percentage of cells with VCN > 10 decreased quickly over time (for most patients until or almost 0%), while the percentage of cells with VCN < 2 concurrently increased. There was no considerable difference between patients treated recently or years ago. Nevertheless, in some patients (e.g. from the compassionate use programme), a high percentage of cells with VCN > 10 remained *in vivo*. Although long-term data from -treated patients (up to 7 years) have not shown the *in vivo* outgrowth of certain cell clones and malignancies, the applicant will continue to monitor insertional mutagenesis and oncogenesis in the treated patients. It is fully endorsed that individual patients are closely and long-term monitored, especially considering that it is not clear what will happen in patients in which higher percentages of VCN > 10 colonies will persist.

Reproduction and developmental toxicity

Dedicated reproduction and developmental studies were not undertaken. As the transduction of the cells occurs *ex vivo*, the risk for transduction of the gametes and consequently the risk from germline transduction is limited and rather theoretical. The applicant addressed the risk of secondary transduction, which appears very limited upon the use of clinical-grade viral vector and the presence of human serum. Bystander cell transduction, or the potential for vector mobilisation, was not observed to occur *in vivo* in animals, further downgrading the risk of transduction of gametes. Although distribution of ARSA LV transduced $CD34^+$ cells to the testis was observed, transfer of the transgene to the male gametes is thus not foreseen. There is no information on the distribution of ARSA LV transduced CD34+ cells in the ovaria, but it is regarded that the absence of bystander cell transduction holds also true for the female gametes. The absence of dedicated studies addressing the reproductive and developmental toxicity can thus be agreed upon.

Taken together, the (long-term) toxicity studies did not indicate existence of safety issues related to transplantation with ARSA LV transduced autologous murine Lin^- or human $CD34^+$ HSPCs, other than issues that are to be expected with 'normal' HSPC transplantation. The evaluation of the insertion site analysis data revealed that a mutagenic risk that may have the potential to result in oncogenic transformation related to the treatment of autologous cells with ARSA-LV is estimated to be very low and can be considered rather theoretical. Nevertheless, individual patients will be closely and long-term monitored to further follow-up potential efficacy- or safety-related concerns.

ERA

According to the applicant's assessment, the outcome of the environmental risk assessment is a negligible risk for human health and the environment. It is agreed that the risks to the environment related to the transduced cells are negligible. It is also agreed that the risk to the environment of the residual infectious particles in the product can be considered negligible. Overall, with the implementation of the control measures described by the applicant the overall risks for human health are considered negligible.

The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.5.6. Conclusion on non-clinical aspects

Pharmacodynamic studies investigating the treatment of MLD mice through transplanting murine equivalent HSPCs transduced with ARSA-LV show proof of principle for the proposed clinical treatment. However, insight in the VCN-ARSA relationship and the relationship between minimal ARSA levels in brain and the efficacy of the treatment cannot be fully provided (because of lack of individual murine data) and these potential relationships are not to be directly translated to the clinic. Supportive information on the VCN-ARSA relationship, the minimal ARSA activity requirement for clinical efficacy and for efficacious treatment in different patient populations is expected to be part of the quality and clinical modules.

The pharmacokinetic studies with either mouse equivalent cells in wild type or diseased mice or with human cells in immune-compromised animals show that genetically modified ($CD34^+$) HSPCs distribute and repopulate also the brain. Animal data suggest that this occurs most efficiently upon busulfan myeloablative pre-conditioning. Furthermore, the ARSA enzyme is not likely to be transduced over the BBB. Therefore, restoring ARSA activity in the brain is rather dependent on reconstitution of µ-cells from ARSA-LV transduced-and-engrafted HSPCs. These cells express and secrete functional ARSA that can also cross-correct the disease phenotype in endogenous neurons and glial cells (that have taken up ARSA from the transduced cells) as is shown in $As2^{-/-}$ mice transplanted with ARSA-HA LV Lin-HSPCs.

The toxicity studies do not indicate safety concerns associated with the treatment with ARSA-LV transduced HSPCs. Transduction of HSPCs with ARSA seems not to reveal an oncogenic risk, but long-term follow-up of the treated MLD patients will be needed.

Vector mobilisation from human to mouse cells was not observed in mice transplanted with ARSA LV-transduced human CB-derived $CD34^+$ cells, confirming that ARSA-LV distributed stably associated with the human genome and is not transferred into genomes of the transplanted mice cells, suggesting a low risk of mobilization and transduction of bystander cells, including germ cells.

It is agreed with the applicant, that this product poses a negligible risk for human health and the environment.

From a non-clinical point of view, there are no objections against a MA.

The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.6. Clinical aspects

2.6.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the Community

were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- *Tabular overview of clinical studies*

Study ID	Study Design and population	Libmeldy (OTL-200) formulation; dose; Busulfan conditioning	Objective/ endpoint
201222 (Registrational Study)	Nonrandomised, open-label-, prospective, comparative (non-concurrent control), single centre study 20 subjects; 9 LI, 11 EJ[d]	OTL-200-f; 2-20 x 10^6 $CD34^+$ cells/kg; *SMAC*[a]: Subjects treated prior to Jan 2014 (9 subjects) *MAC*[b]: Subjects treated after Jan 2014 (11 subjects)	Primary endpoints: Total GMFM score (2 years) ARSA Activity (total PBMCs, 2 years) Secondary endpoints: ARSA activity (BM and CSF), NCV, Brain MRI, GMFC-MLD, Neuropsychological tests, Neurological evaluations, Survival, Engraftment (LV transduced cells, VCN)
CUP 207394	Single patient CUP (Expanded Access Programmes) 1 subject (EJ)	OTL-200-f; 2-25 x 10^6 $CD34^+$ cells/kg; *SMAC*[a]	
HE 205029	(Expanded Access Programmes) 3 subjects (LI)	OTL-200-f; 2-20 x 10^6 $CD34^+$ cells/kg; *SMAC*[a]: 1 subject *MAC*[b]: 2 subjects	
CUP 206258	(Expanded Access Programmes) 5 subjects; 4 LI; 1 EJ	OTL-200-f; 2-30 x 10^6 $CD34^+$ cells/kg; *SMAC*[a]: 2 subjects *MAC*[b]: 3 subjects	
205756	Non-randomised, open-label, single centre 4 subjects; 2 LI, 2 EJ	OTL-200-c; 3-30 x 10^6 $CD34^+$ cells/kg; *MAC* (n=4)[c]	

2.6.2. Pharmacokinetics

No formal PK assessment has been performed, this is acceptable. The reflection paper on stem cell-based medicinal products (*EMA/CAT/571134/2009*) points out that the effect of different doses/cell numbers should be addressed during the nonclinical phase and confirmed during the clinical studies.

PK assessments were performed for the conditioning regimens and will be discussed below.

Exposure busulfan conditioning

The conditioning regimen initially implemented in the OTL-200-f clinical development programme consisted of 14 doses of busulfan (according to subject's weight; submyeloblative conditioning regimen (SMAC)). Subsequently, the conditioning regimen was modified with the goal of reducing the variability of transduced cell engraftment and designed to produce a higher cumulative busulfan AUC. This new conditioning regimen consisted of body surface area-based dosing of busulfan according to the subject's age (myeloablative conditioning regimen (MAC)). In the Integrated Safety Set, 13 subjects (45%) were treated with a SMAC regimen, defined as a target cumulative AUC of 67,200 µg*h/L (target range 58,800 to 78,400 µg*h/L). Sixteen subjects (55%) were administered the MAC regimen, defined as a target cumulative AUC of 85,000 µg*h/L (target range: 76,500 to 93,500 µg*h/L).

For SMAC busulfan plasma levels were monitored by serial pharmacokinetic (PK) sampling and adjusted using a target dose area under the curve (AUC) of 4800 µg*h/L (range: 4200 to 5600 µg*h/L), which corresponds to an expected total cumulative AUC of 67,200 µg*h/L (range 58,800 to 78,400 µg*h/L). Busulfan plasma levels were monitored by timed sampling following the first and the 5th or 6th dose (depending on PK-based dose adjustment requirements). The 5th and subsequent doses of busulfan were adjusted using the area under the curve (AUC), derived after the first dose. An additional adjustment at the 9th or 10th dose could be made depending on the AUC value derived after the 5th or 6th dose. For MAC the second and subsequent doses were adjusted based on the AUC derived after the first dose. The adjustment was performed using a target total cumulative AUC of 85,000 µg*h/L (range: 76,500 to 93,500 µg*h/L). A further adjustment could be made at the third dose based on the AUC values derived from the second dose.

As it would be expected, subjects who received a SMAC regimen received a lower total dose (mg) and lower total dose per body weight (mg/kg) than subjects who received a MAC regimen (Table 1).

The disease subtypes were disproportionately represented in the subgroup who received the SMAC regimen; 9 of 13 subjects (69%) were LI subjects versus 4 of 13 EJ subjects (31%).

Table 1: Busulfan Conditioning, by Regimen (Integrated Safety Set)

Parameter, Summary Statistic	SMAC Regimen (N=13)	MAC Regimen (N=16)	Total (N=29)
Total Dose (mg), Geometric Mean (95% CI)	146.680 (114.194, 188.407)	204.302 (162.740, 256.479)	176.102 (148.600, 208.694)
Total Dose (mg), Median (Min, Max)	155.300 (72.00, 268.00)	221.150 (108.96, 408.00)	162.500 (72.00, 408.00)
Total Dose (mg), %CVb	43.27	44.70	46.96
Total Dose/kg (mg/kg), Geometric Mean (95% CI)	12.666 (11.425, 14.042)	15.555 (13.261, 18.246)	14.186 (12.816, 15.704)
Total Dose/kg (mg/kg), Median (Min, Max)	13.400 (9.00, 16.20)	15.540 (10.37, 31.75)	14.010 (9.00, 31.75)
Total Dose/kg (mg/kg), %CVb	17.19	30.63	27.19

Abbreviations: CI=confidence interval; CVb=coefficient of variation between subjects; MAC=myeloablative conditioning; Max=maximum; Min=minimum; SMAC=sub-myeloablative conditioning

The mean cumulative AUC in subjects who received a SMAC regimen was higher than expected (geometric mean 71,923.53; 95% confidence intervals [CIs]: 68,751.04, 75,242.41), but the mean remained within the target range (Table 2). Two subjects received SMAC regimens but had AUCs within the MAC range (84,305 µg*h/L and 78,572 µg*h/L). The mean cumulative AUC in subjects who received a MAC regimen was consistent with the target AUC (geometric mean 84,043.08; 95% CI: 75,543.86, 81,314.89).

There was low variability in busulfan AUC for both SMAC and MAC regimens (CV 7.5% and 3.5%, respectively). The average exposure to the MAC regimen was 14% higher than the average exposure to the SMAC regimen.

Table 2: Busulfan Total AUC (Integrated Safety Set)

Summary Statistics **(µg*h/L)**	SMAC Regimen (N=13)	MAC Regimen (N=16)	Total (N=29)
Geometric Mean (95% CI)	71,923.53 (68,751.04, 75,242.41)	84,043.08 (82,369.52, 85,750.65)	78,376.28 (75,543.86, 81,314.89)
Median (Min, Max)	70,841.00 (63,420.0, 84,305.0)	84,987.00 (78,000.0, 88,310.0)	79,940.00 (63,420.0, 88,310.0)
% CVb	7.5	3.8	9.7

Abbreviation: AUC=area under the curve; CVb=coefficient of variation between subjects; CI=confidence interval; MAC=myeloablative conditioning; max=maximum; min=minimum; SMAC=sub-myeloablative conditioning

The cumulative AUC in 2 subjects who received a SMAC regimen was outside the target range; therefore, the applicant also analysed data by total AUC. When exposure was defined in the conditioning regimens based on the total AUC threshold of 76,500 µg*h/L, a larger proportion of subjects (18 subjects, 62%) received a total AUC >76,500 µg*h/L compared with the proportion of subjects with AUC threshold ≤76,500 µg*h/L (11 subjects, 38%) (Table 3).

Table 3: Busulfan Conditioning, by Total AUC Threshold of 76,500 **µg*h/L** (Integrated Efficacy Set)

Parameter, Summary Statistic	**≤76,500 µg*h/L** (N=11)	>76,500 **µg*h/L** (N=18)	Total (N=29)
Total dose (mg), Geometric mean (95% CI)	147.972 (109.310, 200.307)	195.865 (158.878, 241.463)	176.102 (148.600, 208.694)
Median (Min, Max)	156.300 (72.00, 268.00)	186.310 (108.96, 408.00)	162.500 (72.00, 408.00)
CVb (%)	47.47	44.02	46.96
Total dose/kg (mg/kg), Geometric mean (95% CI)	12.770 (11.371, 14.342)	15.128 (13.049, 17.539)	14.186 (12.816, 15.704)
Median (Min, Max)	13.400 (9.00, 16.20)	14.810 (10.37, 31.75)	14.010 (9.00, 31.75)
CVb (%)	17.40	30.40	27.19

Abbreviations: CI=confidence interval; CVb=coefficient of variation between subjects; MAC=myeloablative conditioning; Max=maximum; Min=minimum; SMAC=sub-myeloablative conditioning

In Study 205756 of OTL-200-c, all 4 subjects received a MAC regimen. The total dose of busulfan received ranged from 11.48 mg/kg to 14.56 mg/kg. The mean observed cumulative AUC in these subjects was 80009.25 µg*h/L (range: 79965, 80058 µg*h/L), showing little variation from the target exposure.

2.6.3. Pharmacodynamics

No dedicated pharmacodynamics study was performed, instead various pharmacodynamic parameters were measured in all clinical studies of Libmeldy. These include engraftment of transduced cells (in bone marrow (BM), peripheral blood mononuclear cells [PBMCs] and cell subpopulations) and ARSA activity (in bone marrow, in PBMCs and cell subpopulations, and in cerebrospinal fluid [CSF]).

The Integrated efficacy data set includes data from subjects treated with the fresh OTL-200-f formulation in the registrational Study (Study 201222 [n=20], the core data set) and patients treated under the 3 Expanded Access Programmes (n=9). The subjects included were diagnosed with either LI MLD or EJ MLD and could be symptomatic or pre-symptomatic (See clinical efficacy for a more detailed description of the population).

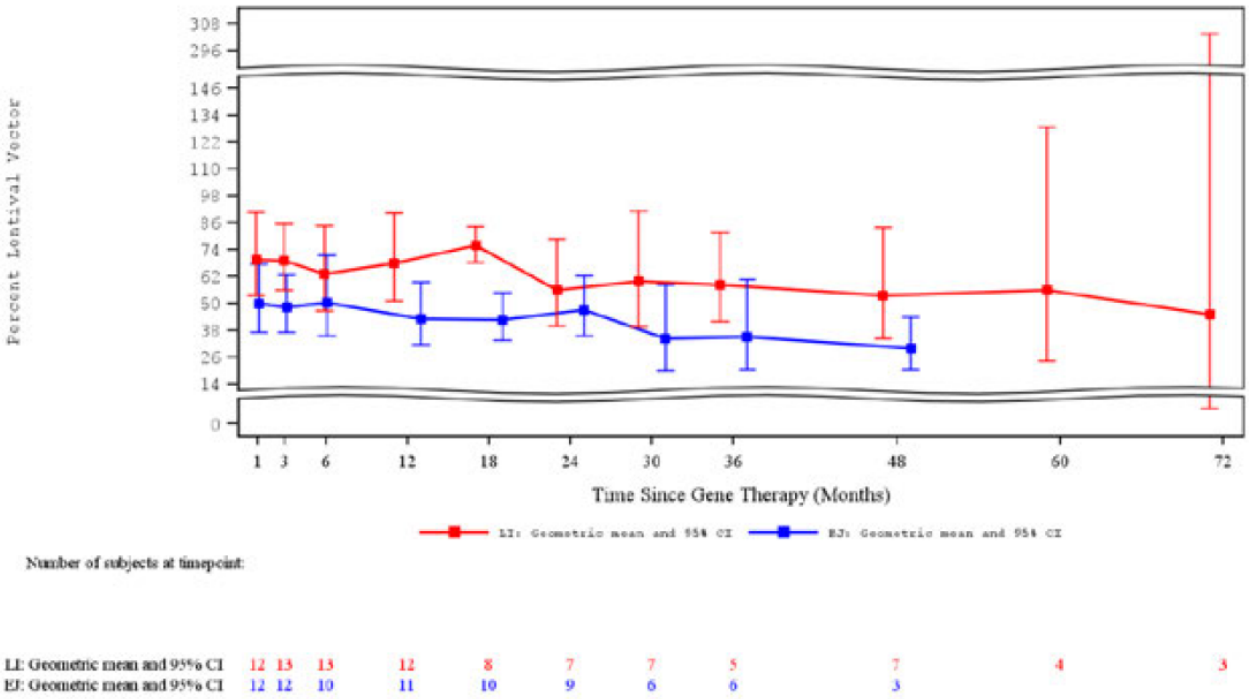
The design of the pivotal study and expanded access programmes was comparable, e.g. Screening phase, Baseline phase, Treatment phase and Follow Up phase. The target dose was 5×10^6 to 10×10^6 CD34+ cells/kg (minimum dose 2×10^6 CD34+ cells/kg, maximum dose 20×10^6 CD34+ cells/kg), dependent on the yield of cells following DP manufacturing. The source material was either collected from bone marrow harvest or mobilised peripheral blood apheresis. The doses given varied within the pre-defined dose range in the study protocol.

Transduced Cell Engraftment in BM-Derived Clonogenic Progenitor Cells

Across the OTL-200-f Integrated Efficacy Set, the proportion of BM-derived colonies harboring the LV genome (percentage LV+) at Year 1 after treatment was 54.8% (range 20% to 100% [n=23]). At Year 5, that proportion was 45.0% (range 18.8% to 90.6% [n=6]).

At all timepoints evaluated, geometric mean LV+ values cells in BM were higher in the LI subgroup than in the EJ subgroup, although the 95% CIs overlapped (See Figure 2).

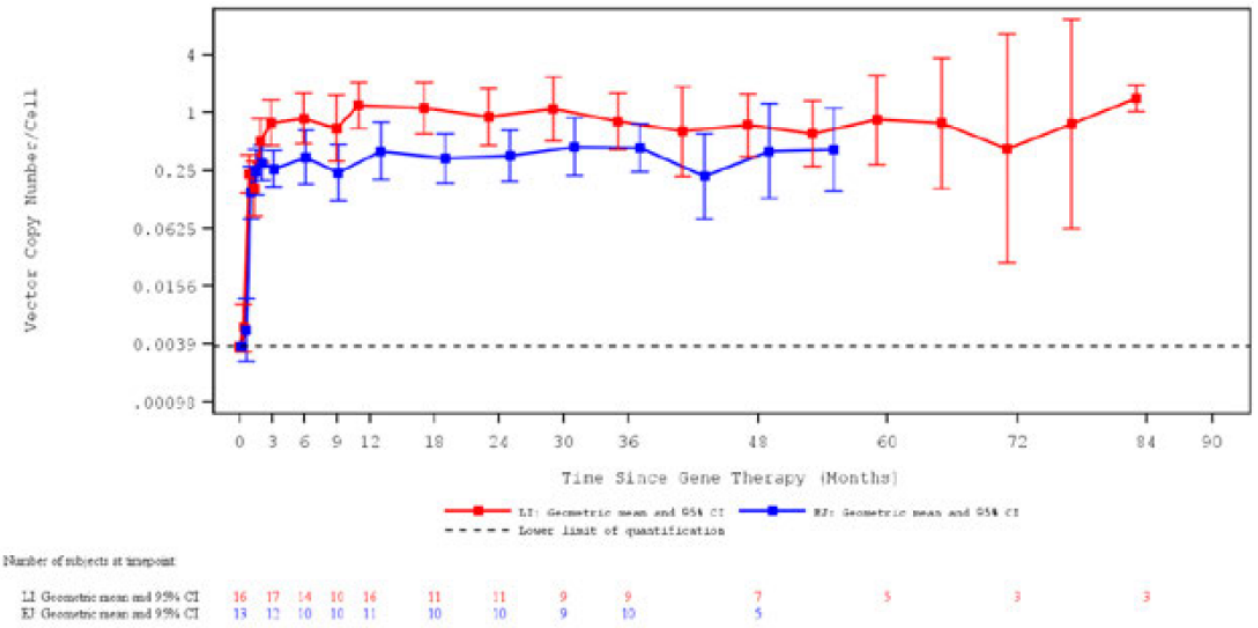
Figure 2: Percentage of Lentiviral Vector Transduced Cells in Bone Marrow Over Time (Geometric Mean and 95% CI), OTL-200-f Treated Subjects by Disease Subtype (Integrated Efficacy Set)



VCN values in total PBMCs also indicated engraftment of transduced cell beginning at 28 days post treatment. The mean value was mean 0.19 copies/cell [range 0.03 to 0.68] (n=29), thus above the minimum target defined in the protocol, i.e., ≥0.04 copies/cell, equivalent to 4%.

In both the LI and EJ subgroups, the VCN in total PBMCs initially increased over time and remained relatively stable from 3months post-treatment throughout the course of follow-up (See Figure 3)

Figure 3: Vector Copy Number in PBMCs Over Time (Geometric Mean and 95% CI), OTL-200-f Treated Subjects by Disease Subtype (Integrated Efficacy Set).



Note: Lower limit of quantification (LLOQ) is 0.0037 VCN/cell. Values less than the LLOQ were imputed as the LLOQ. Zero values are plotted as plotted at 0.001. Geometric means and 95% CIs are presented where there are at least 3 subjects with non-missing data.

ARSA activity

Measurement of the reconstitution of ARSA activity in the haematopoietic system was performed on PBMCs, BM MNC, and other PB and BM subpopulations. ARSA activity in CSF was also quantified to provide indirect evidence that transduced cells have migrated to the CNS and are producing and secreting functional ARSA enzyme.

ARSA Activity in Total Peripheral Blood Mononuclear Cells

The ARSA activity in the PBMCs increased within 1 month and at 3 months was at levels higher than reported for healthy subjects. At Year 2 post treatment (the time of the primary endpoint), a statistically significant increase in ARSA activity in total PBMCs for both the LI (18.7-fold increase; 95% CI: 8.3, 42.2; p<0.001) and EJ (5.7-fold increase; 95% CI: 2.6, 12.4; p<0.001) subgroups compared to baseline levels was observed. The statistically significant increase in ARSA activity in total PBMCs also remained in both the LI (37.5-fold increase; 95% CI: 17.7, 79.6; p<0.001) and EJ (11.2-fold increase; 95% CI: 5.7, 21.9 p<0.001) subgroups compared with Baseline levels at Year 3.

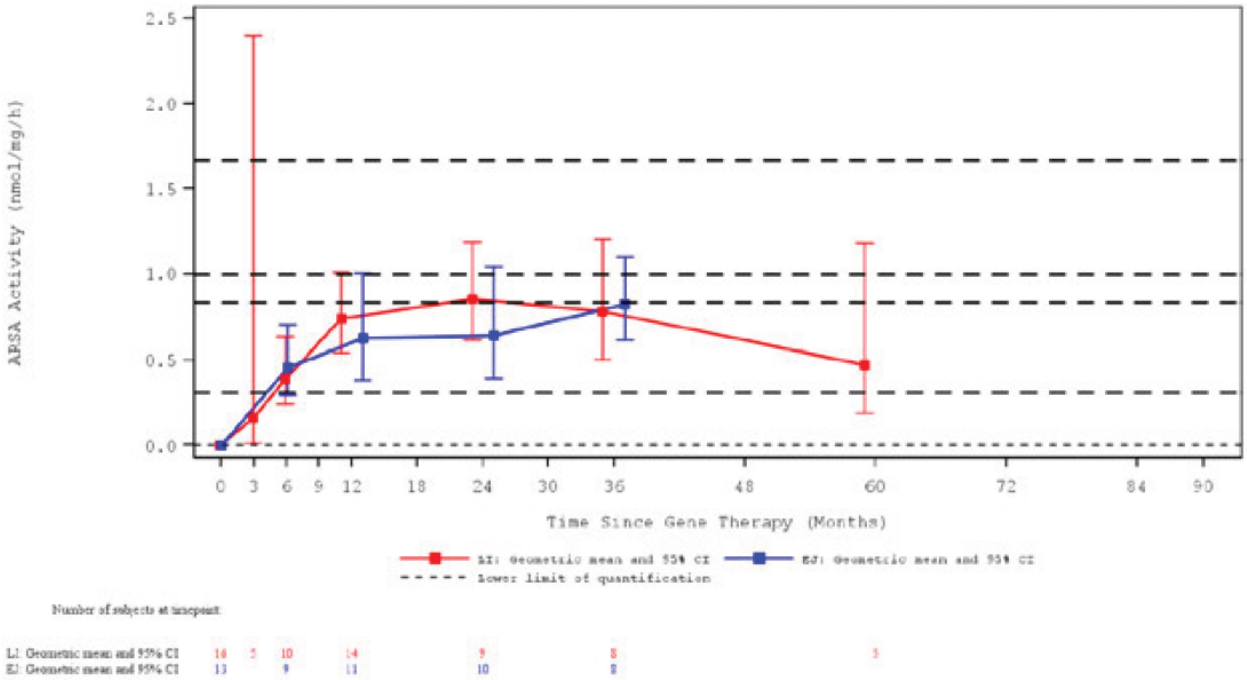
ARSA Activity in MNC

Consistent with ARSA activity in PBMCs, mean ARSA activity levels in total BM MNC also increased substantially within 1 month after treatment. By 3 months post treatment, mean ARSA activity levels in total BM MNC were 7.5-fold higher than Baseline in LI subjects and 6.3-fold higher than Baseline in EJ subjects (p<0.001 for all). Throughout the course of follow-up, mean ARSA levels in total BM MNC remained at least 7.5-fold and at least 5.3-fold higher than Baseline in LI and EJ subjects, respectively.

ARSA Activity in CSF

At Baseline, ARSA activity levels in CSF in all subjects were below the LLOQ (0.0032 nmol/mg/h). following treatment, ARSA activity levels in all subjects were detectable by Month 6, with a mean level of 0.42 nmol/mg/h (range 0.13 to 102). At Year 1 post treatment the mean ARSA activity in the CSF was 0.739 nmol/mg/h for the LI MLD group and 0.473 nmol/mg/h for the EJ MLD group. Although there is a fluctuation in CSF ARSA activity levels, the activity levels remain within ranges reported for healthy subjects up to 5 years post treatment (Figure 4).

Figure 4: ARSA Activity in Cerebrospinal Fluid Over Time (Geometric Mean and 95% CI), by Disease Subtype (Integrated Efficacy Set)



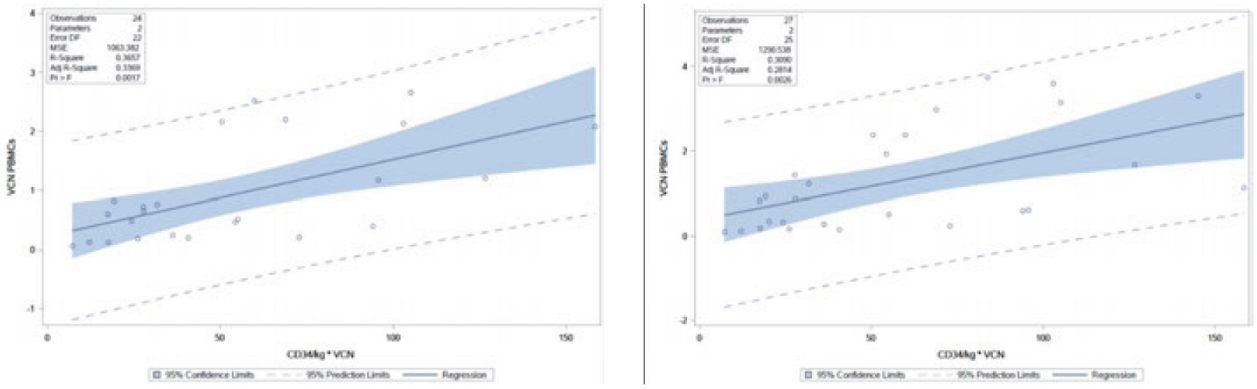
Note: Geometric mean and 95% CI were presented where there were at least 3 subjects with non-missing data.
Note: The reference range represents data from a cohort of paediatric reference donors as per Perugia reference range report.

Correlation curves

Specific focus was on the correlations of CD34+/kg dose multiplied by VCN (CD34+ * VCN) as a measure of product potency.

At 6 months and 12 months post-treatment, statistically significant correlations between CD34+ * VCN and VCN in total PBMCs were observed (corr=0.605, p=0.002; corr=0.556, p=0.003 at 6 months and 12 months respectively) (Figure 5).

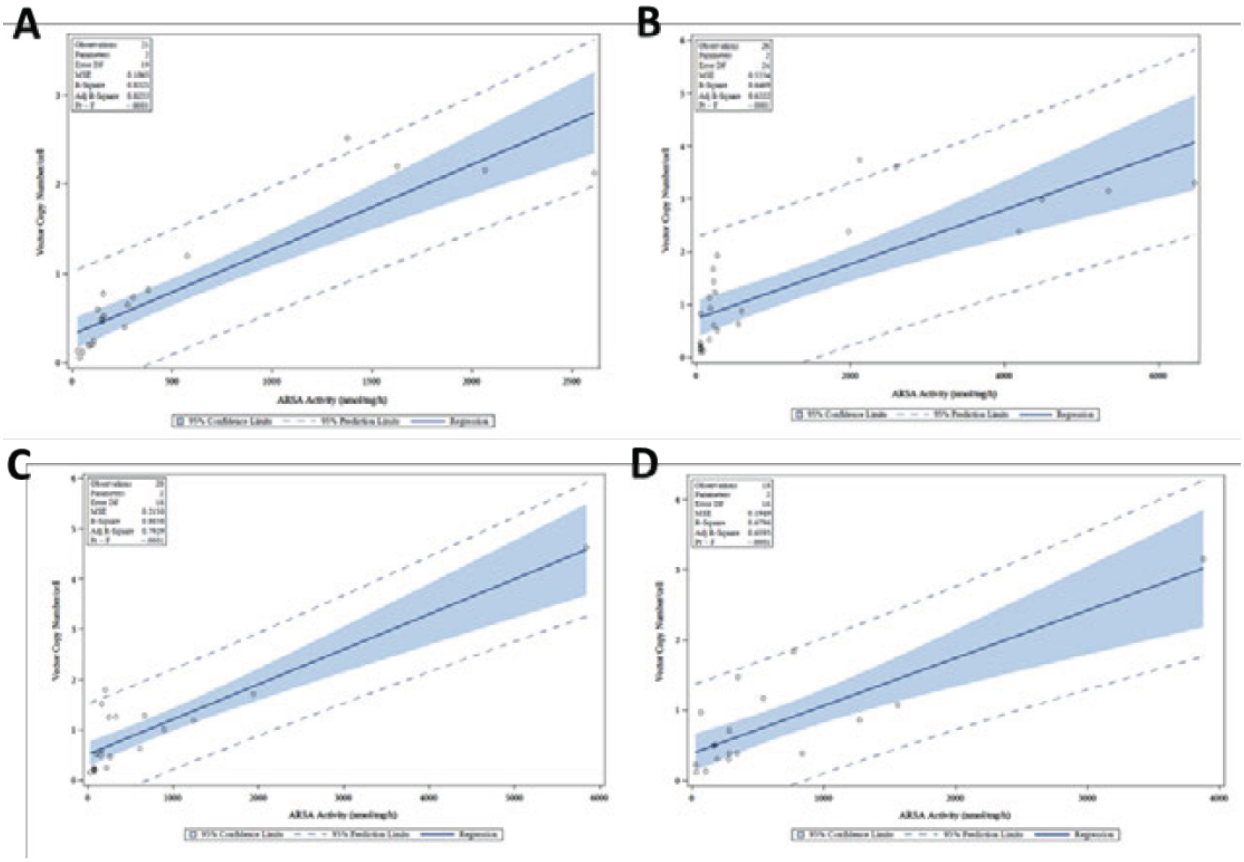
Figure 5: Scatterplot of VCN in PBMCs versus CD34+cells/kg * VCN at 6 Months and 1 Year Post-Treatment (Integrated Efficacy Set)



The applicant indicates that no relevant correlations were observed between CQA of OTL-200-f DP and clinical biomarkers at 2 years and 3 years post-treatment.

When clinical samples at different timepoints post gene-therapy were evaluated for potential relationships between mean VCN and ARSA activity in total PBMCs, statistically significant correlations between VCN in PBMCs and ARSA activity in PBMCs were observed at 6 months, 1 year, 2 years and 3 years post-treatment (see Figure 6).

Figure 6: Scatterplot of VCN in PBMCs versus ARSA activity in PBMCs at Months (A), 1 Year (B), 2 Years (C) and 3 Years (D) Post-Treatment (Integrated Efficacy Set)



Abbreviations: ARSA=arylsulfatase A; PBMC=peripheral blood mononuclear cells; VCN=vector copy number.

Note by Assessor: The information on the axes and legends are not very clear. In all Figures the ARSA Activity is presented on the X-axes and the VCN on the Y-axes. The X-axes ranges from 0-2500 (500 interval), 0-6000 (2000 interval), 0-6000 (1000 interval) and 0-4000 (1000 interval) for panel A, B, C and D respectively. On the Y-Axes in al panels, the interval is 1. The soled thick line is the regression line, the blue area is the confidence interval of the regression curve and the dotted line indicates the 95% prediction limits.

No relevant correlations were observed between levels of ARSA activity in CSF and motor function, cognition or MRI total scores in any of the MLD variant and timepoints evaluated (2 Years and 3 Years post treatment) (see Table 4). The applicant concludes that due to lack of a correlation the minimal ARSA activity level in CSF to achieve efficacy and maintenance of effect could not be determined.

Table 4: Correlations Between ARSA in CSF and Clinical Efficacy Outcomes

	ARSA in CSF					
	Year 2			**Year 3**		
	Pre-symptomatic Late Infantile	**Pre-symptomatic Early Juvenile**	**Early-symptomatic Early-Juvenile**	**Pre-symptomatic Late Infantile**	**Pre-symptomatic Early Juvenile**	**Early-symptomatic Early-Juvenile**
GMFM total score	n=9 p=0.229 corr.=0.446	n=4 p=0.983 corr.=0.017	n=6 p=0.053 corr.=-0.806	n=8 p=0.317 corr.=0.407	n=3 p=0.146 corr.=0.974	n=5 p=0.283 corr.=-0.602
GMFC-MLD	n=9 p=0.262 corr.=-0.419	Not calculable[1]	n=6 p=0.029 corr.=0.858	n=8 p=0.346 corr.=-0.385	Not calculable[1]	n=5 p=0.229 corr.=0.656
DQ	n=9 p=0.936 corr.=0.031	n=4 p=0.871 corr.=0.29	n=6 p=0.027 corr.=-0.863	n=6 p=0.423 corr.=0.407	n=3 p=0.700 corr.=-0.454	n=5 p=0.055 corr.=-0.869
MRI	n=9 p=0.420 corr.=-0.308	n=4 p=0.819 corr.=-0.181	n=6 p=0.139 corr.=0.677	n=8 p=0.523 corr.=-0.266	n=3 p=0.325 corr.=0.873	n=5 p=0.197 corr.=0.690

[1] Not calculable due to all pre-symptomatic EJ subjects scoring GMFC-MLD Level 0 with variable levels of ARSA in CSF (Figure 2.7.3.3.2.117)

Source: Figure 2.7.3.3.2.111

Conditioning regimen

Transduced cell engraftment by conditioning regimen

A similar percentage of LV-transduced HSPCs (or their progeny) was measured over time in BM after MAC and SMAC or when the conditioning subgroups were defined based on an AUC threshold of 76,500 µg*h/L (see clinical AR for figures). In addition, there was no clear correlation between busulfan exposure, as measured by total AUC, and the proportion of LV-positive cells (Spearman correlation coefficient 0.117).

There were no differences in the level of transduced cell engraftment, as measured by VCN in BM and PBMCs, in the subgroups of subjects who received MAC vs. SMAC or when the conditioning subgroups were defined

based on an AUC threshold of 76,500 µg*h/L (see clinical AR for figures). In addition, there was no clear correlation between busulfan exposure and the VCN per cell in BM or PBMCs (Spearman rank correlation coefficient: -0.015 and -0.048, respectively).

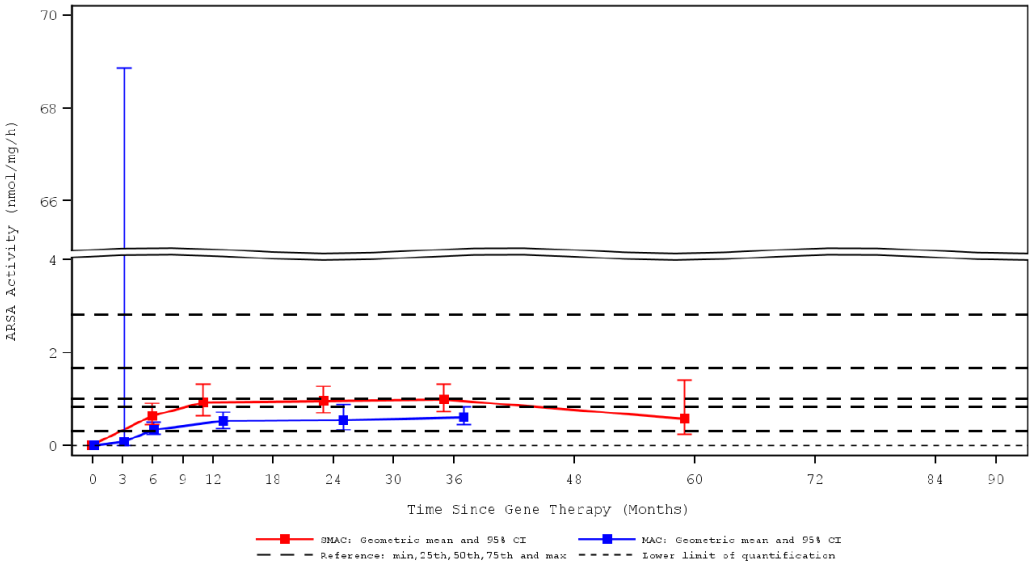
ARSA activity by conditioning regimen

The increase in ARSA activity in PBMCs at Year 2 was similar in the subgroup of subjects who received SMAC (10.4-fold [range 1.00 to 226.56]) and in the subgroup of subjects who received MAC (10.7-fold [range 2.70 to 75.33]). Similar increases were also observed in BM-derived MNCs at Year 2 (SMAC: 6.4-fold [range 1.5 to 21.8]); MAC 11.0-fold [range 4.74 to 63.76])

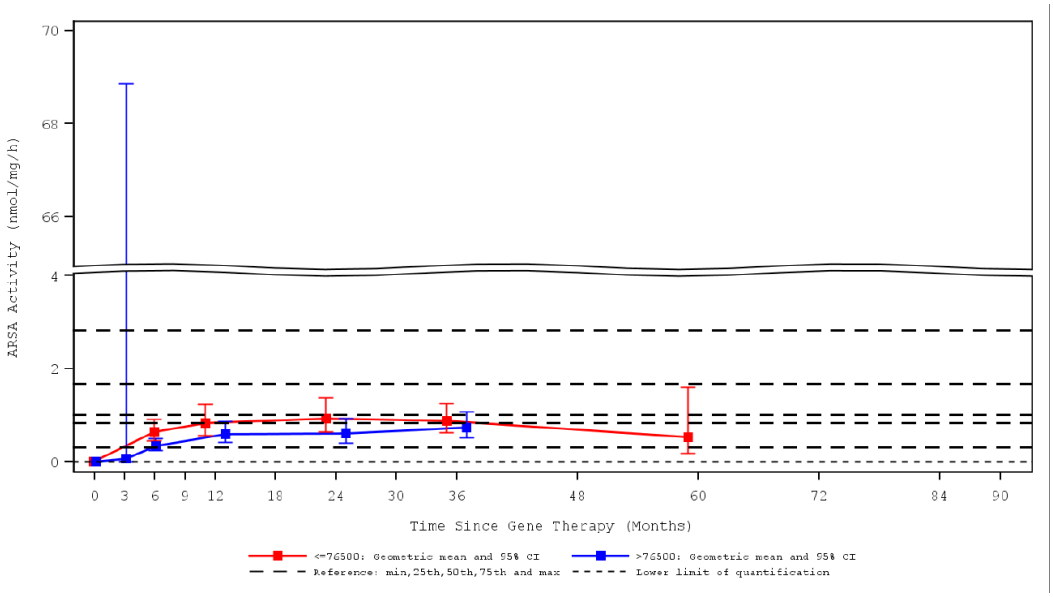
In CSF, mean ARSA activity was slightly higher in the SMAC subgroup at Year 2 (geometric mean 0.954 nmol/mg/h [range 0.60 to 1.99 nmol/mg/h]) than in the MAC subgroup (geometric mean 0.547 nmol/mg/h [range 0.13 to 0.92 nmol/mg/h]). This difference was smaller when the conditioning subgroups were defined as total AUC threshold of ≤76,500 µg*h/L vs. >76,500 µg*h/L (1.5-fold difference between Year 2 geometric means of 0.934 nmol/mg/h [range 0.60 to 1.99] and 0.615 nmol/mg/h [range 0.13 to 1.06 nmol/mg/h] respectively) (Figure 7).

Figure 7: ARSA Activity in CSF Over Time (Geometric Mean and 95% CIs), OTL-200-f Treated Subjects by Conditioning Regimen (SMAC vs. MAC [Panel A]; Total AUC Threshold 76,500 **µg*h/L** [Panel B])

Panel A. SMAC vs. MAC



Panel B. Total AUC ≤76,500 µg*h/L vs. >76,500 µg*h/L



Abbreviations: LLOQ = lower limit of quantitation; MAC = myeloablative conditioning; SMAC = sub-myeloablative conditioning
Notes: Values of 0 are plotted at 0.001. Values below the LLOQ are imputed at LLOQ. LLOQ is 25.79 nmol/mg/h.
Geometric means and 95% CIs are presented where there are at least 3 subjects with non-missing data.
The reference range represents data from a cohort of adult reference donors as per TIGET validation report [Module 5.3.1.4, OSR/SR-TIGET document number T TCL 009-0].

2.6.4. Discussion on clinical pharmacology

A conventional clinical pharmacology programme (dose escalation/dose range finding, human absorption, metabolism and excretion, drug-drug interaction, and special population studies) for Libmeldy was not considered feasible, which is agreed. A range of doses, various pharmacodynamic (PD) and pharmacokinetic (PK) parameters were measured in the clinical studies of Libmeldy.

Pharmacokinetics

Two different regimens were used in the clinical development programme. Thirteen patients (45%) were treated with SMAC (submyeloablative conditioning regimen) with a median AUC of 70,841.00 µg*h/L (min 63,420.0; max 84,305.0). Two subjects (Patient 1 and 2) received SMAC regimens but had AUCs within the MAC range. The PK monitoring and dose adjustments used for these two subjects were based on a target single AUC. Next, the protocol was adjusted to reduce the risk of busulfan overexposure observed in these two subjects . In the adjusted protocol monitoring was based on a total cumulative target AUC. Afterwards, all subjects conditioned with either regimen (MAC or SMAC) have shown total cumulative AUC within the pre-specified protocol range. Sixteen patients (55%) received the MAC (myeloablative regimen) and all patients had an AUC within the target range. Median AUC was 84,987.00 µg*h/L (min 78,000.0; max 88,310.0). The average exposure to the MAC regimen was 14% higher than the average exposure to the SMAC regimen. The disease subtypes (LI MLD and EJ MLD) where disproportionally represented in the different regimen groups.

Furthermore, the dose levels differed between the subgroups. It is not possible to conclude with the data available if different dose levels would have been required with different disease subtypes.

Pharmacodynamics

The engraftment of lentiviral vector positive cells is higher in both LI MLD and EJ MLD than the predefined threshold of 4%. The engraftment of the lentiviral vector positive cells remained stable over time. At 2 years post treatment there was an overlap in engraftment of the total mononuclear cells in the bone marrow, between the LI and EJ subvariants. Although the geometric mean VCN/cell was higher for the LI subvariant (0.815 [95% CI 0.422; 1.577]) than for the EJ subvariant (0.463 [95%CI 0.254; 0.843]) (total MNC in BM). It is unclear if this is related to the switch from SMAC to MAC busulfan regimen during the development of the clinical programme, as there is an imbalance in representation of SMAC and MAC. A similar effect, e.g. sustained engraftment of lentiviral vector positive cells irrespective of disease variant, was also observed for peripheral blood mononuclear cells [PBMCs] and cell subpopulations (e.g. CD34+).

Supra physiological ARSA enzyme activity levels were observed in PBMCs, and BM in both LI MLD and EJ MLD subjects treated with Libmeldy. The ARSA activity levels in the CSF were similar to that of healthy subjects.

The plots showing a correlation between CD34+cells/kg * VCN and VCN in PBMCs should be interpreted with caution considering that the majority of show a VCN in PBMCs between 0 and 1, irrespective of dose administered. The same caution should be applied for the presented correlation between VNC in PBMC and ARSA activity in PBMC. Insufficient data is available above PBMC VCN levels of 1 to enable definite conclusions. Nevertheless, clinically it appears that even the lowest VCN achieved in this trial is sufficient to provide ARSA activity levels in the CSF similar to what has been reported for healthy subjects.

The applicant modified the conditioning regimen from SMAC to MAC to reduce variability of transduced cell engraftment. The rationale for the applicant to change the conditioning regimen was based on preliminary analysis of the data from the first 9 patients enrolled in Study 201222 and treated with the SMAC regimen. Whilst engraftment was sustained in all of these subjects, the level of transduced cells engraftment was variable with values ranging from 25% to 80% of LV positive forming cells in BM. In addition, ARSA activity seemed to be positively correlated with engraftment values, with ARSA activity reaching normal and above normal values in patients with the highest transduced cell engraftment. In order to improve the therapeutic potential of Libmeldy on both the CNS and PNS by reducing transduced cell engraftment variability and increasing engraftment levels, the applicant modified the conditioning regimen (to MAC regimen) with the aim to increase busulfan exposure by approximately 10% of the total AUC. Both the percentage of LV-transduced cells in BM and VCN in BM and PMBCs were overlapping between the SMAC and MAC regimen, although the sample sizes are small. There is therefore no conclusive evidence that the use of MAC leads to higher engraftment levels. Also ARSA activity in total PBMCs and BM over time did not seem to differ between SMAC vs MAC. For both SMAC and MAC ARSA levels in CSF reached normal values after Libmeldy treatment. Interestingly, for ARSA activity in CSF the curves for SMAC and total AUC ≤76,500 µg*h/L are above the curves for MAC and total AUC >76,500 µg*h/L. Definitive conclusions are, however, difficult to make due to the small patient numbers and the imbalance in clinical subtype of patients treated with SMAC or MAC regimen with a disproportionate representation of LI subjects in the SMAC subgroup. Although the data in the Libmeldy studies do not show clear differences in terms of engraftment efficiency and ARSA activity between SMAC and MAC, the applicant prefers to use the MAC regimen in clinical practice. This is based on literature showing that in children and young adults treated with allogenic haematopoietic cell transplantation for a variety of malignant and non-malignant disorders a target cumulative AUC of busulfan between 78,000-101,000 µg*h/L provides the busulfan exposure-response relationship with optimal efficacy. Furthermore, higher levels of myeloablative conditioning obtained with MAC are preferable in neurometabolic disorders

according to the applicant to enable an intense brain conditioning to successfully remove resident microglia and favour high central engraftment of genetically modified cells.

Based on the provided data it is not possible to have one of the regimens as preferred choice and section 5.1 of the SmPC describes that the choice is up to the treating physician based on an individual benefit/risk assessment.

No correlation was found between ARSA activity CSF level and any of the clinical outcome measures examined, i.e. GMFM, GMFC-MLD,DQ and MRI. The applicant indicates that the minimum ARSA activity level in the CSF required for efficacy could not be determined, due to lack of a correlation. This is not agreed. Figure 13 below shows that although the subjects performed better than subjects from the NHx cohort, deterioration was observed in motor function assessed by GMFM in per-symptomatic LI-MLD subjects who had ARSA activity levels of 07.1nmol/mg/hr and 0.37nmol/mg/hr. Therefore, levels below 0.71nmol/mg/hr could be indicative for treatment effect. It is unclear if this could also be the threshold for EJ-MLD subjects as that group consisted of pre-symptomatic, early-symptomatic and symptomatic subjects. The ARSA activity levels required for an effect for these stages may be different. The relationship between CSF protein content and ARSA activity levels will be further established post marketing. This will allow evaluation of ARSA CSF activity as predicting factor for treatment success or explanation of failure.

2.6.5. Conclusions on clinical pharmacology

The engraftment parameter for lentiviral vector positive cells set by the applicant, i.e. >4% is met, irrespective of disease subvariant, busulfan conditioning, dose used, or formulation used. Supra-physiological ARSA activity values are measured in the PBMC and MNCs, however from non-clinical data it is known that supratherapeutic levels do not pose a risk.

It should be noted that for the majority of the subjects the ARSA activity within the CSF after treatment was within ranges reported for healthy subjects.

The clinical pharmacology data were considered adequate to support the application.

The CHMP endorse the CAT assessment regarding the conclusions on the Clinical pharmacology as described above.

2.7. Clinical efficacy

Dose-response studies and main clinical studies

No formal dose response study was performed. The applicant based the dose on doses described in literature. The applicant has improved the yield of CD34+ cells harvest and mobilization and also the dose administered range from $2.0x10^6$ – $20x10^6$ CD34+cells/kg to a range of $3.0x10^6$-$30x10^6$ CD34+cells/kg, during the course of the clinical programme.

The proposed dose range i.e. number of transfected cells can be safely administered, as this was done for several other studies using LV. However, there is no direct correlation between VCN and ARSA activity levels in the CSF. This issue is not pursued as ARSA activity in all measured sources, e.g. PBM, CSF, cell type etc., are within or above the normal range.

It should also be noted that non clinical data indicate that ARSA activity levels higher than reported for healthy subjects do not lead to unfavourable effects.

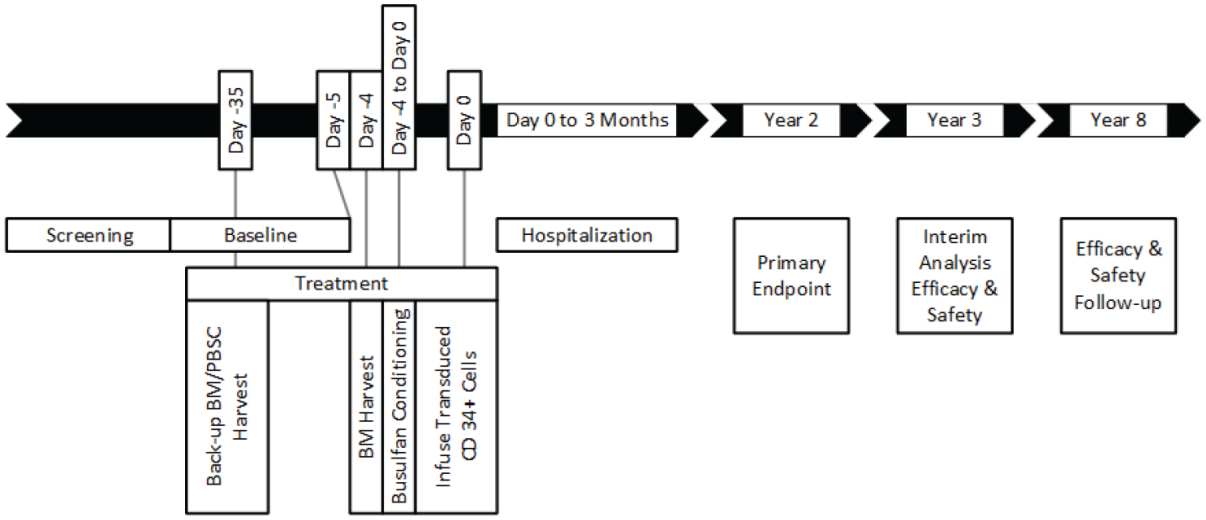
Main study

Registration Study/ Study 201222

Methods

Study 201222 was a non-randomised, open-label-, prospective, comparative (non-concurrent control), single centre study. The study consisted of 4 phases: 1) Screening phase (evaluation in/exclusion criteria), 2) Baseline phase, 3) Treatment phase (cell harvest for investigational DP manufacture on day -4, busulfan conditioning (Day -4 to Day -1, administration of LIBMELDY on Day 0) and 4) a Follow up phase (8 years) (see Figure 8).

Figure 8: Study Diagram



The overlap between the Baseline and Treatment phases allows for the requirement of performing the Baseline clinical and instrumental evaluations used to assess the efficacy of the treatment (e.g., GMFM scale and brain MRI) at the latest possible time before treatment.

Study Participants

A total of 22 MLD subjects, 9 LI MLD and 13 EJ MLD confirmed by ARSA enzymatic activity and genetic analysis were included in the study. All LI study subjects and some EJ subjects were identified after an older sibling had developed symptoms and received an MLD diagnosis, prompting the testing of other family members.

All 9, LI subjects were all pre-symptomatic upon enrolment, defined as subjects without neurological impairment or without symptoms or signs of MLD, however, one subject became symptomatic prior to treatment with Libmeldy.

For the EJ MLD, 4 subjects were pre-symptomatic and 8 were early-symptomatic defined as subject identified within 6 months from the first reported symptoms or subjects meeting with an intelligence quotient (IQ) ≥70 and the ability to walk independently for ≥10 steps.

Comparator population

Data from TIGET NHx Study consists of a cohort of 31 untreated LI and EJ MLD subjects. The data contain a mixture of cross-sectional and longitudinal data with some subjects contributing data at multiple time points while other providing data from a single visit, possibly because subjects may not have been able to travel for clinic visits due to disease progression or may have died prior to providing longitudinal data.

Matched sibling data was available for 9 subjects treated in study 201222.

Treatments

The planned minimum dose was 2 × 10^6 CD34+ cells/kg, with a target of 5 × 10^6 to 10 × 10^6 $CD34^+$ cells/kg (maximum dose 20 × 10^6 $CD34^+$ cells/kg), dependent on the yield of cells following DP manufacturing.

Busulfan conditioning regimen

Rationale conditioning regimen

The conditioning regimen initially implemented in the Libmeldy-f clinical development programme consisted of 14 doses of busulfan (according to subject's weight). Subsequently, the conditioning regimen was modified with the goal of reducing the variability of transduced cell engraftment and designed to produce a higher cumulative busulfan AUC. This new conditioning regimen consisted of body surface area-based dosing of busulfan according to the subject's age. Both conditioning regimens will be discussed in more detail below.

Submyeloblative conditioning regimen (SMAC)

Subjects prescribed a SMAC regimen received body weight-based doses of IV busulfan according to the schematic in Table 5.

Table 5: Busulfan Dosage According to Subject's Weight (SMAC Regimen)

Body Weight (kg)	<9	9 to <16	16 to 23	>23 to 34	>34
Busulfan dosage (mg/kg/dose)	1	1.2	1.1	0.95	0.8

Subjects received a total of 14 doses, given as a 2-hour infusion administered every 6 hours from Day -4 to Day -1. Busulfan plasma levels were monitored by serial pharmacokinetic (PK) sampling (see PK section) and adjusted using a target dose area under the curve (AUC) of 4800 µg*h/L (range: 4200 to 5600 µg*h/L), which corresponds to an expected total cumulative AUC of 67,200 µg*h/L (range 58,800 to 78,400 µg*h/L).

In the registrational study (201222), subjects enrolled prior to January 2014 (9 subjects) were treated with this SMAC regimen. Four EAP Patients (Patient 22, 24, 25, 29) also received the SMAC regimen.

Myeloablative conditioning regimen (MAC)

Subjects prescribed a MAC regimen received a body surface area (Mosteller's formula)-based dose of busulfan according to the subject's age (Table 6).

Table 6: Busulfan Dosage According to Patient's Age (MAC Regimen)

≤1 year of age	>1 year of age
80 mg/m^2/dose	120 mg/m^2/dose

Under this MAC regimen, subjects received a total of 4 doses, administered as a 3-hour IV infusion every 20 to 24 hours from Day -4 to Day -1. PK monitoring is described in the PK section.

Subjects treated after January 2014 (11 subjects) in the registrational study (Study 201222) received this MAC regimen. Five patients in the EAPs (Patient 21, 23, 26, 27, 28) were also administered the MAC regimen. All 4 subjects enrolled in Study 205756 at the time of data cut received the MAC regimen.

Objectives

The objectives of this ongoing study are as follows:

- Evaluation of the safety of Libmeldy-f in MLD subjects, considering both the conditioning regimen safety and the safety of LV-transduced cell administration, short- and long-term after the treatment
- Evaluation of the efficacy of Libmeldy-f, assessed as reduction in the progression of the clinical motor impairment in treated subjects compared to the progression measured in untreated MLD subjects within the Telethon Institute for Gene Therapy (TIGET) Natural History (NHx) Study (TIGET NHx Study; Study number 204949), accompanied by a significant increase of residual ARSA activity as compared to subjects' pre-treatment values. Motor functions will be measured by the clinically relevant GMFM scoring system

Outcomes/endpoints

Clinical efficacy was primary based on the Gross Motor Function Measure score (GMFM). The total GMFM score two years after treatment was the primary endpoint. A delay in progression of 10% in total of the total GMFM score in treated subjects as compared to a concurrent historical control group was the aimed effect size.

The co-primary endpoint was the ARSA activity (PBMC and CSF). A *significant (≥2 SD) increase in residual ARSA activity* measured in the BPBMC at two years as compared to pre-treatment values, was the aimed effect size.

Secondary endpoints included nerve conduction velocity (NCV), brain magnetic resonance imaging (MRI), gross motor function classification (GMFC-MLD, neuropsychological tests, neurological evaluations, survival, engraftment (lentiviral vector transduced cells, vector copy number [VCN]). The study included comparison to a natural history cohort (NHx) and where available untreated siblings.

Randomisation and blinding (masking)

Not Applicable

Statistical methods

The All Subjects population includes all subjects enrolled in Study 201222 and subjects enrolled in Study

204949 (TIGET NHx Study) with disease subtype LI or EJ.

The TIGET NHx population includes all subjects enrolled in the TIGET NHx Study with disease subtype LI or EJ and who are not also enrolled in Study 201222.

The Intent-to-Treat (ITT) population included any subject enrolled into Study 201222 who met the inclusion criteria at Screening, had a signed informed consent form (ICF), and had received treatment with Libmeldy-f.

The Matched Analysis Set (MAS) population includes subjects in the ITT population and any age and MLD variant-matched untreated subjects from TIGET NHx Study who provided control data for comparison purposes. Matched untreated participants are defined as subjects with LI or EJ MLD or clinical variant of intermediate severity between the classical LI and EJ forms in the TIGET NHx Study who had a study visit where their age (at the study visit) fit within the window of ages for Libmeldy-f-treated subjects in Study 201222. For each MLD subtype (i.e., LI or EJ), at the 2-year and 3-year analysis time points, the lower bound of the age window was based on the lowest age of a treated subject in Study 201222 minus 3 months and the upper bound was the highest age of a treated subject in Study 201222. For example, if the age range at the 2-year post-GT visit of the treated subjects in the LI subgroup was 43 to 55 months, then any LI participant from the TIGET NHx Study with a visit where their age was between 40 and 55 months was included. If more than 1 visit occurred within the age window and had non-missing data, then the earliest visit was used.

The Matched Sibling Analysis Set population includes subjects in the ITT population who had an untreated sibling in the TIGET NHx Study and included the corresponding untreated sibling(s) from the TIGET NHx Study.

The Safety population includes any subject enrolled in Study 201222 who received Libmeldy-f.

The study population analyses were based on the All Subjects population, unless otherwise specified. Summaries were generated by LI and EJ subgroups in addition to the overall total, unless otherwise specified.

Efficacy Analysis

The efficacy analyses were based on the populations defined above and on evaluations at Year 2, with efficacy evaluations also performed at Year 3.

Primary efficacy endpoints

The total GMFM score two years after treatment was the primary endpoint. The co-primary endpoint was the ARSA activity (in PBMC). A *significant (≥2 SD) increase in residual ARSA activity* measured in the PBMC at two years as compared to pre-treatment values, was the aimed effect size.

The GMFM data at Year 2 and 3 were analysed, for the LI and EJ subgroups separately, using an analysis of covariance (ANCOVA) model fitting age and treatment (Libmeldy-f or TIGET NHx). Age was fitted in months for the LI subjects and in years for the EJ subjects.

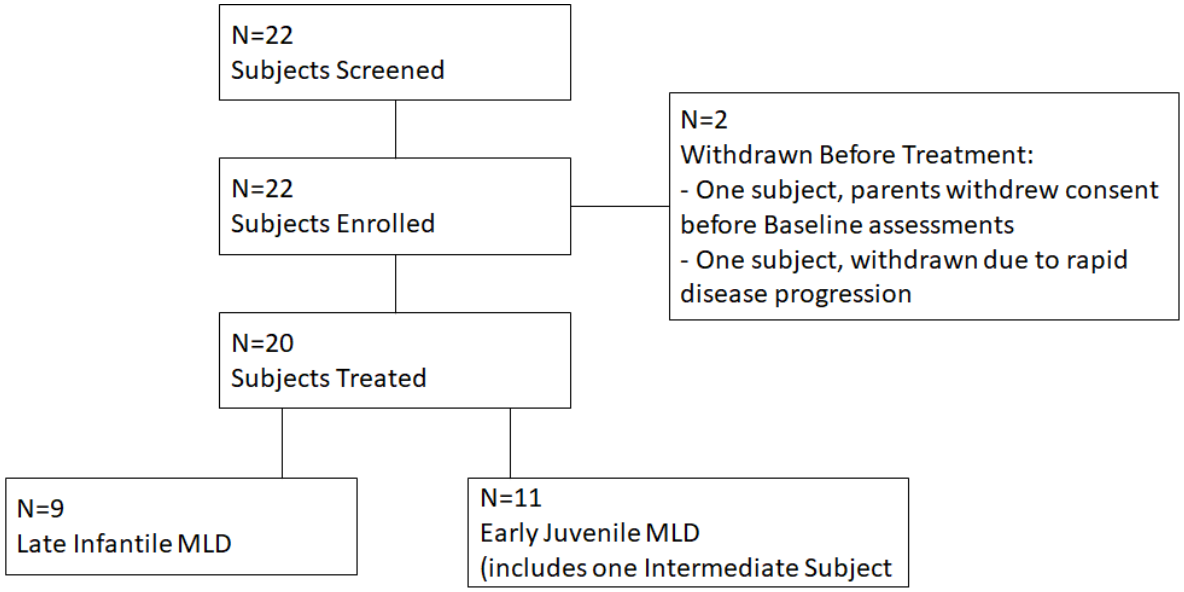
The data variability may be small for the TIGET NHx group since they are likely to be in the advanced stage of the disease and have lower GMFM scores. For the treated subjects, if treatment is effective, the scores could be better and show larger variability. Therefore, the variability for treated and for TIGET NHx groups were estimated from the model separately with different parameters.

ARSA activity measured in total PBMC after treatment was compared with the pre-treatment values. ARSA summaries for all visits and ARSA ratio relative to the Baseline visit at all post-Baseline visits were presented by disease subtype and overall, and separately by busulfan conditioning regimen. The ARSA log transformed data were analysed using a mixed-model repeated measures (MMRM) model fitting Baseline, visit, visit by Baseline interaction, disease subtype, and disease subtype by visit interaction as fixed effects and back transformed to produce estimates in the original scale. The adjusted LS means, the 95% CI of the adjusted LS means, the adjusted LS mean ratio, the 95% CI for the adjusted LS mean ratio, and the p-value for the ratio will be presented for each visit in order to assess if there has been a statistically significant increase in the ARSA activity from Baseline. The ratio from Baseline is considered statistically significant if the associated p-value is <0.05.

Participant flow

As shown in Figure 9, a total of 22 subjects were screened and enrolled into Study 201222. Two of these 22 subjects were withdrawn from the trial prior to treatment. One EJ subject was withdrawn by the investigator at the Baseline visit due to rapid disease progression. The second subject withdrew consent prior to treatment.

Figure 9: Study Participant Flow



Numbers analysed

The Intent-to-Treat (ITT) population includes any subject enrolled into Study 201222 who met the inclusion criteria at Screening, had a signed informed consent form (ICF), and had received treatment with Libmeldy-f.

The study included a total of 22 subject, 9 LI-MLD subjects (8 presymptomatic at time of infusion), 11 EJ-MLD subjects (4 subjects presymtomatic at time of infusion) and 2 EJ-MLD subjects withdrew before treatment. The one symptomatic subject in the LI group became symptomatic just prior to treatment.

Outcomes and estimation

BASELINE DATA

Late infantile (LI) MLD

The summary of Demographics and Baseline Characteristics of LI MLD subjects is presented in Table 7. The mean age of MLD diagnosis for the LI subgroup was 8.7 months, the mean predicted age of onset was 20.6 month and the median age at the time of treatment was 15.0 months (Table 7).

Table 7: Late Infantile: Summary of Demographics and Baseline Characteristics of Individuals in the Late Infantile Subgroup in Study 20122

Subject Number	1	2	3	4	5	6	7	8	9	TOTAL***
Demography and MLD diagnosis information										
Gender	male	male	male	Female	female	male	female	female	Male	Female 4 (44); Male 5 (56%)
Age (months)	15	13	7	17	12	16	23	9	8	Mean 14.10; min 7.6; max 23.3
ARSA mutation 1[b]	c.827C>T (p.Thr276Met)	c.736C>T (p.Arg246Cys)	c.449C>G (p.Pro150Arg)	c.465+1G>A (splice donor)	c.465+1G>A (splice donor)	c.465+1G>A (splice donor)	c.1108-2A>G (splice acceptor)	c.736C>T (p.Arg246Cys)	c.937C>T (p.Arg313*)	N/A
ARSA mutation 2[b]	c.827C>T (p.Thr276Met)	c.737G>A (p.Arg246His)	c.449C>G (p.Pro150Arg)	c.980-1G>A (splice acceptor)	c.855-1G>A (splice acceptor)	c.465+1G>A (splice donor)	c.1108-2A>G (splice acceptor)	c.737G>A (p.Arg246His)	c.937C>T (p.Arg313*)	N/A
Genotype	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0/0
Predicted age of onset, months[c]	18	24-27	15	19	15-18	20	26	24-27	24-30	20.6
Sibling , survival, months (status)	61.3 (died)	68.4 (died)	42.4 (died)	51.8 (withdrawn)	74.7 (died)	Not enrolled	75.8 (alive)	68.4 (died)	Not enrolled	N/A
Baseline characteristics										
Symptomatic	No[d]	No	No	No	No[d]	No[d]	No[d]	No	No[d]	8 PS, 1 S
ARSA activity in PBMCs nmol/mg/h[e]	3.27	10.92	3.17	5.13	16.67	NA	9.85	4.23	2.98	min 2.98; max 16.67
Total GMFM score (%)	65.02	75.63	27.33	80.11	74.99	66.09	71.09	50.92	20.86	min 20.86; max
GMFC-MLD level[f]	NA	NA	NA	NA	NA	NA	1	NA	NA	max 1
NCV Index	-9.79	-0.47	-3.38	-0.16	-6.06	-6.02	-3.11	-1.28	-4.86	min -0.16; max-9.79
Total MRI score	0	0	0	0	0	0	2.25	0	0.25	Min 0 max2.25
Intelligence quotient										
Performance	95	115	100	105	95	100	80	95	95	80-115

Subject Number	1	2	3	4	5	6	7	8	9	TOTAL***
Language	NA	83	112	109	109	94	89	127	106	83-127
Busulfan conditioning										
Regimen	SMAC[g]	SMAC[g]	SMAC	SMAC	SMAC	SMAC	MAC	MAC	MAC	6 SMAC 3 MAC
Exposure – total AUC (µg x h/L)	84,305	78,572	69,225	68,914	70,744	71,551	87,940	78,000	84,990	Min 68,914 Max87,940
CD34+ HSPC dose (x10^6 cells/kg)	11.1	7.0	7.2	4.2	6.2	18.2	13.1	19.5	13.1	Min 4.2, Max 19.5
DP VCN**	2.5	2.5	4.4	1.7	3.1	4.0	4.2	4.3	7.3	2.5 – 7.3

*=stop codon. DP VCN= Drug product vector copy number, PS= pre-symptomatic, S= symptomatic, NA= not assessed, N/A= not applicable
a. Age at administration of Libmeldy-f.
b. Mutations are described according to standard HGVS nomenclature, as described in Listing 2.06.
c. For pre-symptomatic subjects, the predicted age of onset was calculated on the basis of the age at symptom onset in the subject's older sibling(s).
d. Five Subjects (Patient 1,5,6,7 and 9) had abnormal neurological exam findings at Baseline as outlined in Section 5.5.3.
e. LLQ identified post interim study report as 25.79 nmol/mg/h for PBMCs.
f. Note that on a population basis, the GMFC-MLD score cannot be applied before the age of 18 months, as it is based upon the ability to walk. For an individual person who had already started walking before 18 months of age, however, the GMFC-MLD score can be reasonably applied.
g. All subjects except for 2 (Patient 1 and 2)received a total AUC within the acceptable range. These two subjects received a total AUC higher than the acceptable range.
Source: Listing 1.05, Listing 1.06, Listing 1.08, Listing 1.09, Listing 2.03, Listing 2.04, Listing 2.06, Listing 2.39, Listing 2.40, Listing 2.41, Listing 2.42, Listing 2.43, Listing 2.44, Listing 2.45, and Listing 2.51.
**Row added by Assessor
***colum added by assessor. If averages were not reported by the applicant, minimum and maximum values are presented

Early Juvenile (EJ) MLD

A total of 13 EJ-MLD subjects were included in the trial of which 11 received treatment. The summary of Demographics and Baseline Characteristics of EJ-MLD subjects is presented Table 8 for the 4 pre-symptomatic EJ-MLD subjects and Table 9 for the 7 (early) symptomatic EJ-MLD subjects. The median age at the time of treatment was 66.8 months in the EJ subgroup (range: 18.8 to 139.9 months).

Pre-symptomatic EJ MLD

Among the EJ MLD group, 4 of 11 (36%) subjects were pre-symptomatic at Screening and Baseline (including one subject- Patient 10 , who was considered to be affected by a clinical variant of intermediate severity between the classical LI and EJ forms of MLD) (See Table 8). The mean predicted age of onset was 60.1 months.

One subject (Patient 13)had abnormal neurological exam findings at both Screening and Baseline that consisted of brisk tendon reflexes with clonus in lower limbs and mild cerebellar ataxia with dysmetria bilaterally.

Table 8: Pre-symptomatic Early Juvenile Subgroup: Summary of Demographics and Baseline Characteristics Study 201222

Subject Number	10[a]	11	12	13[b]	TOTAL**
Demography and MLD diagnosis information					
Gender	female	Female	male	male	Female 2, male 2
Age (months)	18	66	48	66	Min 18; Max 66

ARSA mutation 1[c]	c.931G>A (p.Gly311Ser)	c.465+1G>A (splice donor)	c.200C>T (p.Pro67Leu)	c.465+1G>A (splice donor)	N/A
ARSA mutation 2[d]	c.931G>A (p.Gly311Ser)	c.1283C>T (p.Pro428Leu)	c.1283C>T (p.Pro428Leu)	c.1283C>T (p.Pro428Leu)	N/A
Genotype	R / R	0 / R	Not known[e] / R	0 / R	N/A
Predicted age of onset[f], months	24-36	83	61	75	Min 24-36; Max 83
Sibling , survival, months (status)	211 (alive)	147.8 (alive[g])	97 (alive)	104.3 (alive)	N/A
Baseline characteristics					
ARSA activity in PBMCs (nmol/mg/h)[h]	17.86	5.41	4.07	0.69	Min 0.69; max 17.86
Total GMFM score (%)	77.91	97.31	95.73	98.61	Min 77.91; max 97.31
GMFC-MLD level[f]	0	0	0	0	0
Symtomatic	NO	NO	NO	NO	
NCV Index	-10.25	-3.89	-3.07	-3.14	Min 3.14; Max -10.25
Total MRI score	0	3.5	4.25	3.75	Min 0; Max 4.25
Intelligence quotient					
Performance	90	127	124	115	Min 90; Max 127
Language	79	107	130	118	Min 79; Max 130
Busulfan conditioning					
Regimen	SMAC	MAC	MAC	MAC	SMAC 1; MAC 3
Exposure – total AUC (µg x h/L)	73,146	84,988	84,972	84,996	Min 73,146 Max 84,996
CD34+ HSPC dose (x10^6 cells/kg)	16.3	9	9.7	6.7	Min 6.7; Max 16.3
DP VCN**	2.5	3.1	5.6	5.4	Min 2.5; Max 56

a. Classified as an 'Intermediate' clinical variant, not matching the typical LI or EJ forms. Data for this subject have been pooled with EJ dataset for analysis (see Section 5.5.3 for additional details). Note: The current HGVS nomenclature for the homozygous mutation of the subject and his sibling was inadvertently identified as c.925G>A (p.Glu309Lys) in Listing 2.06; the correct HGVS nomenclature is c.931G>A (p.Gly311Ser).
b. Patient 13 had abnormal neurological exam findings at Baseline as outlined in Section 5.5.3.
c. Age at administration of Libmeldy-f.
d. Mutations are described according to standard HGVS nomenclature as described in Listing 2.06.
e. Not known refers to ARSA gene variant where there is insufficient data to assign severity to the allele.
f. For pre-symptomatic subjects, the predicted age of onset was calculated on the basis of the age at symptom onset in the subject's older sibling(s).
g. Symptomatic EJ subject who did not meet the eligibility criteria for Study 201222 and was treated with Libmeldy-f via Compassionate Use (GSK identifier 205029).
h. LLQ identified post interim study report as 25.79 nmol/mg/h for PBMCs.
Source: Listing 1.05, Listing 1.06, Listing 1.08, Listing 1.09, Listing 2.03, Listing 2.04, Listing 2.06, Listing 2.39, Listing 2.40, Listing 2.41, Listing 2.42, Listing 2.43, Listing 2.44, Listing 2.45, and Listing 2.51.
**Row or Colum added by Assessor

Symptomatic EJ MLD

Seven of 11 (64%) EJ subjects were symptomatic from Screening (See Table 9). The mean age of onset for early-symptomatic EJ subjects was 59.8 months. One Subject (Patient 14)showed motor and cognitive dysfunction at Screening and experienced further rapid disease progression between Screening and Baseline; additional information is summarised below (See Table 9).

Table 9: Early Symptomatic Early Juvenile Subgroup: Summary of Demographics and Baseline Characteristics in Study 201222

Subject Number	14	15	16	17	18	19	20
Demography and MLD diagnosis information							
Gender/Race/Age[a], months	Female/ White/59	Male/White/ 38	Female/White/88	Male/White/ 139	Female/White/84	Female/White/69	Female/White/71
ARSA mutation 1 (protein or splice site alteration[b])	c.383T>G (p.Leu128Arg)	c.1150G>A (p.Glu384Lys)	c.465+1G>A (splice donor)	c.465+1G>A (splice donor)	c.1175G>A (p.Arg392Gln)	c.465+1G>A (splice donor)	c.1283C>T (p.Pro428Leu)
ARSA mutation 2 (protein or splice site alteration[b])	c.1283C>T (p.Pro428Leu)	c.1223_1231del9 (p.Ser408_Thr410del)	c.1283C>T (p.Pro428Leu)	c.1283C>T (p.Pro428Leu)	c.1283C>T (p.Pro428Leu)	c.1283C>T (p.Pro428Leu)	c.929delG (p.Gly310Alafs)
Genotype	0 / R	R / 0	0 / R	0 / R	0 / R	0 / R	R / 0
Age at onset, months	54	35	66	64	56	60	65
Sibling survival, months (status)	NA	NA	NA	127.4 (alive)	NA	NA	NA
Baseline characteristics							
ARSA activity in PBMCs (nmol/mg/h)[c]	5.33	18.39	3.45	14.45	27.98	12.04	8.56
Total GMFM score (%)	73.91	87.06	99.44	86.06	86.76	81.24	78.04
GMFC-MLD level	1	1	0	1	1	1	1
NCV Index	-7.58	-4.73	-9.51	-9.27	-8.86	-7.93	-3.17
Total MRI score	11	0.5	8.75	4	12	10	10
Intelligence quotient							
Performance	50	100	119	115	89	82	87
Language	76	103	110	102	104	102	112
Busulfan conditioning							
Regimen	SMAC	SMAC	MAC	MAC	MAC	MAC	MAC
Exposure – total AUC (µg x h/L)	70,506	70,841	84,080	84,986	85,000	85,404	88,310
$CD34^+$ HSPC dose ($x10^6$ cells/kg)	9.9	7.1	6.6	8.9	10	6	11.1

a.Age at administration of OTL-200-f.

b.Mutations are described according to standard HGVS nomenclature as described in Listing 2.06.
c.LLQ identified post interim study report as 25.79 nmol/mg/h for PBMCs.
Source: Listing 1.05, Listing 1.06, Listing 1.08, Listing 1.09, Listing 2.03, Listing 2.04, Listing 2.06, Listing 2.39, Listing 2.40, Listing 2.41, Listing 2.42, Listing 2.43, Listing 2.44, Listing 2.45, and Listing 2.51.

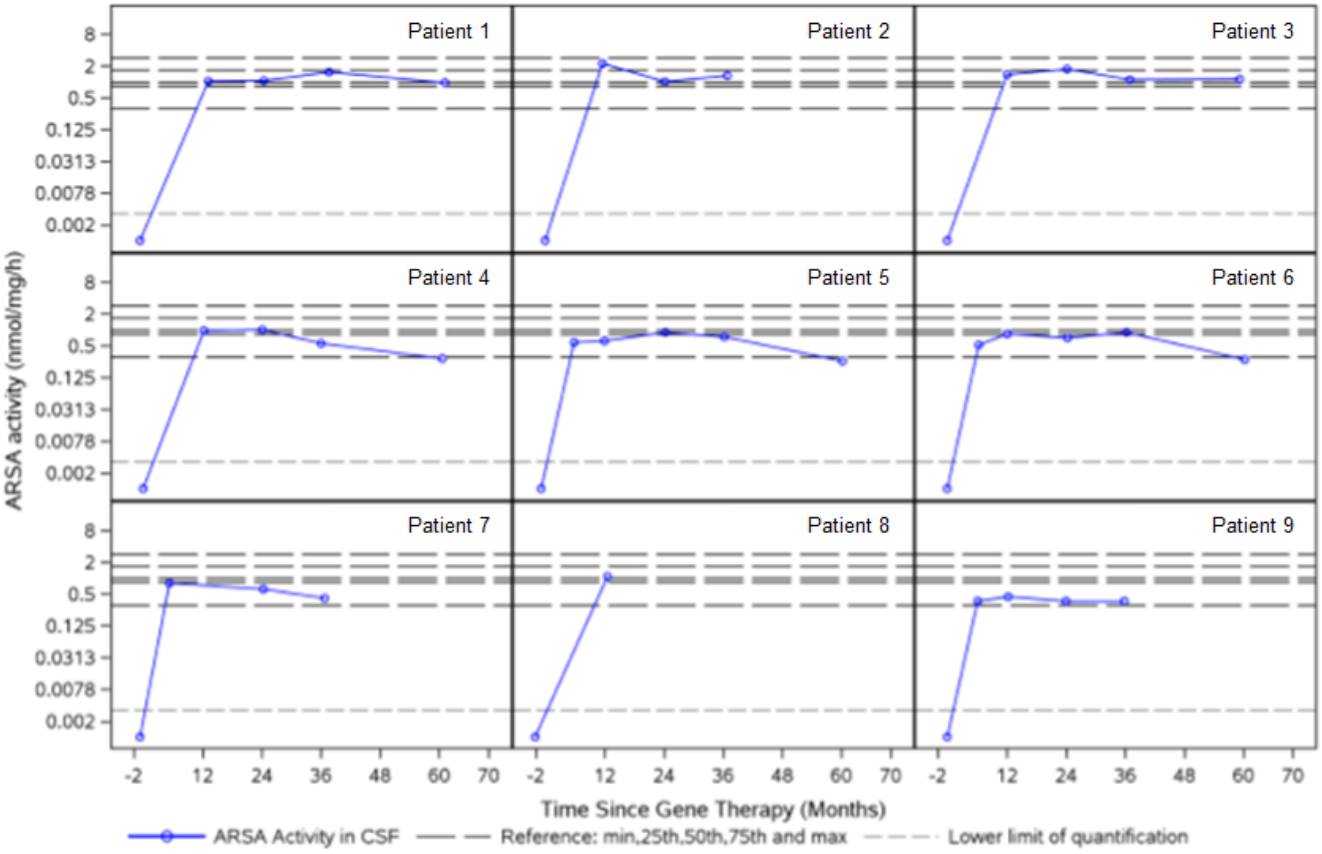
ARSA ACTIVITY IN THE CEREBROSPINAL FLUID

Late Infantile MLD (LI MLD)

At Baseline, ARSA activity levels in CSF in all *LI MLD* subjects were below the LLOQ (0.0032 nmol/mg/h). Post treatment, ARSA activity levels in the CSF were detectable by Month 6 and levels of 0.9745 nmol/mg/h were reached at 1-year post treatment. The average ARSA activity in the CSF for the LI MLD subjects

measured 5 years post treatment was 0.4726 nmol/mg/h. Individual panel plots for the LI MLD group are shown in Figure 10 below.

Figure 10: Late Infantile Subgroup (ITT Population; N=9): Panel Plot of ARSA Activity in Cerebrospinal Fluid Over Time (nmol/mg/h)

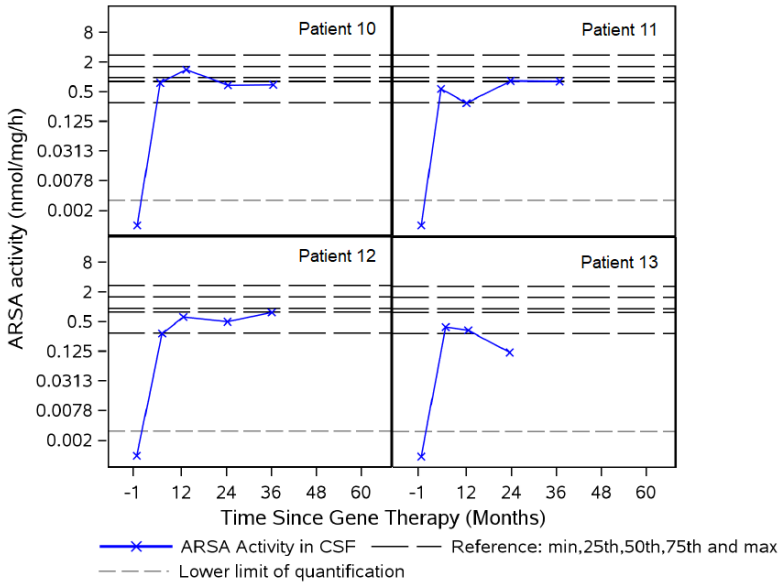


Note: Values ≤0/undetectable ARSA activity were imputed at LLOQ. LLOQ was 0.0032 nmol/mg/h.
Note: The reference range represents data from a cohort of paediatric reference donors as per Perugia reference report.

Early Juvenile (EJ) MLD

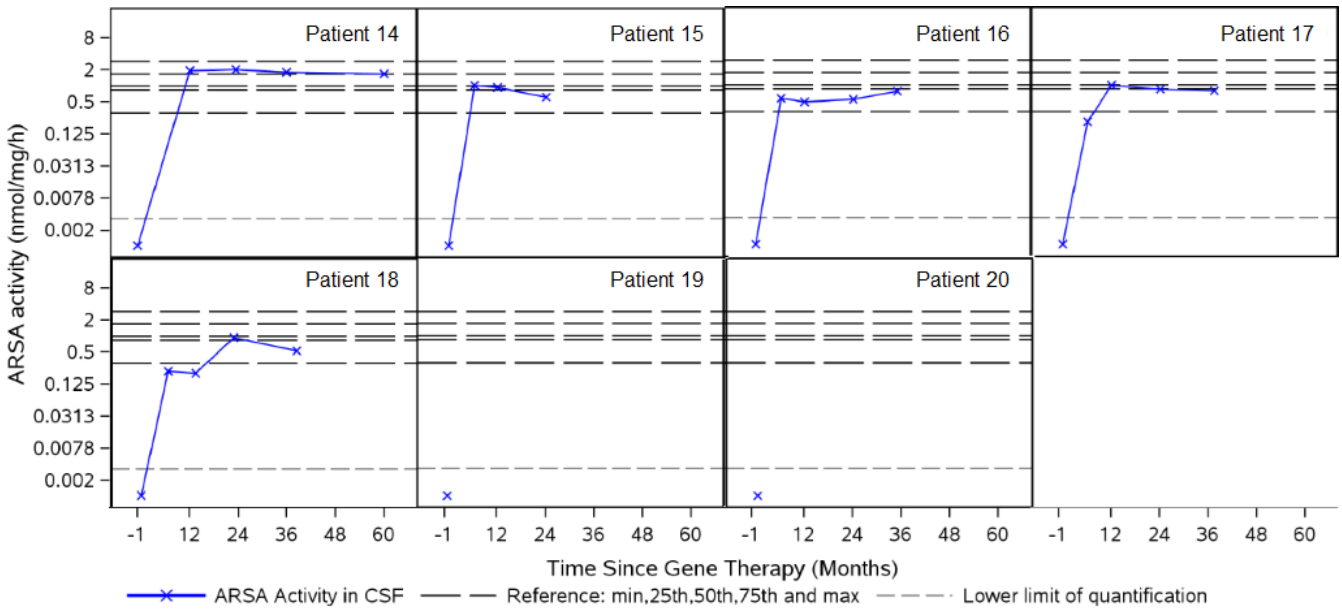
Similar to the effects reported in the *LI MLD* subjects, for the *EJ MLD* subjects the ARSA activity levels in the CSF increased from levels below detection limit to within ranges reported for healthy subjects. The ARSA activity levels in all subjects were detectable by Month 6, with a mean level of 0.47 nmol/mg/h (95% CI: 0.34, 0.65 and a mean level of 0.6352 nmol/mg/h 1-year post treatment. At 5 years post treatment the mean ARSA activity in the CSF was 1.67 nmol/mg/h for the overall EJ group. The individual panel plots for the *pre-symptomatic EJ MLD* subjects is presented in Figure 11 and for the symptomatic EJ MLD subject in Figure 12.

Figure 11: Pre-symptomatic Early Juvanile: Panel Plot of ARSA Activity in Cerebrospinal Fluid Over Time (nmol/mg/h)



Note: Values ≤0 were imputed at LLOQ. LLOQ was 0.0032 nmol/mg/h.
Note: Geometric mean and 95% CI were presented where there were at least 3 subjects with non-missing data.
Note: The reference range represents data from a cohort of paediatric reference donors as per Perugia reference range report.

Figure 12: Early-symptomatic Early Juvenile Subgroup (ITT Population; N=7) Panel Plot of ARSA Activity in Cerebrospinal Fluid Over Time (nmol/mg/h)



Note: Values ≤0 were imputed at LLOQ. LLOQ was 0.0032 nmol/mg/h.
Note: Geometric mean and 95% CI were presented where there were at least 3 subjects with non-missing data.
Note: The reference range represents data from a cohort of paediatric reference donors as per Perugia reference range report.
Note: Four subjects (Patient 10, 11, 12, 13) were pre-symptomatic at Libmeldy-f administration, and seven subjects (Patient 14, 15, 16, 17, 18, 19, 20) were early-symptomatic at Libmeldy-f administration.

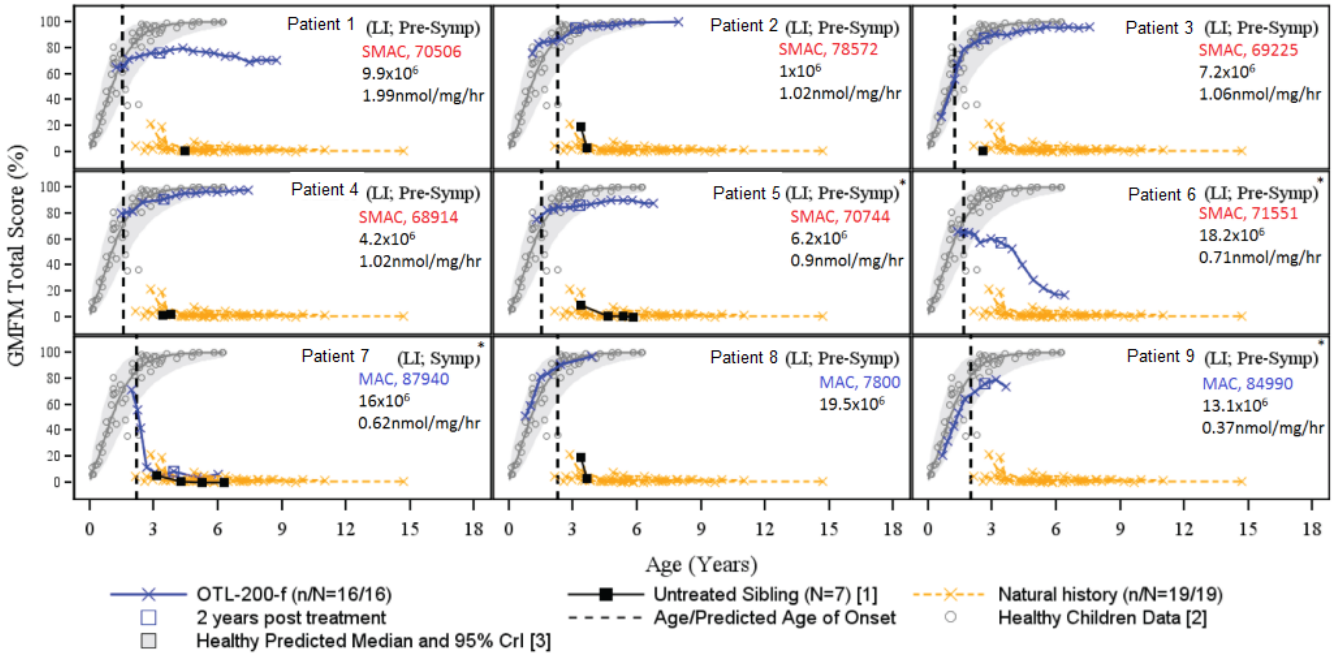
GROSS MOTOR FUNCTION MEASUREMENT SCORE

Late Infantile MLD (LI MLD)

At year 2 post treatment, mean total GMFM score in the Libmeldy treated LI subjects was 72.5 compared to 7.4 for the NHx subjects (Difference 65.1 points, 95%CI 41.6; 88.6), p<0.001).

GMFM total score (%) and domain profiles for MLD subjects who received Libmeldy-f (blue open circles) and untreated MLD subjects enrolled in the TIGET NHx study are presented graphically in Figure 13. A Bayesian credible interval was fitted as a function of age for healthy children and represented by the grey solid area in the figures. The predicted median score for healthy children is represented by the grey line. In addition, each Libmeldy-f-treated subject's predicted or actual age of disease onset is shown as a vertical dotted line.

Figure 13: Late Infantile Subgroup: Panel Plot of Gross Motor Function Measure Total Score (%) Over Time Compared to TIGET NHx Data**



[1] Untreated sibling data is a subset of NHx data.
[2] If there are 2 reference lines for "Age/Predicted Age of Onset," this reflects the range given in the eCRF.
[3] Healthy children data from Dr. Palisano and colleagues, who provided access to the anonymous age and GMFM-88 data on 60 subjects in the "No CP" group as reported in (Palisano, 1997).
**figure adapted by Assessor to include:
- Dose (CD 34+ cells/kg)
- ARSA CSF activity levels indicated measured 2 years post treatment (primary endpoint marked by applicant).
*neurological scores hinting at early progression (Patient 1, 5, 6, 9)
- busulfan conditioning: SMAC and MAC treatment indicated with corresponding AUCs.
Note: As of Protocol Amendment 11, the drug product name has been changed from GSK2696274 to Libmeldy-f.

As shown in Figure 13, gross motor function is clearly higher in the majority of LI subjects treated with Libmeldy-f when compared with their untreated siblings and untreated LI TIGET NHx Study participants. The

majority of treated LI subjects (7/9, 78%) have reached or have been followed up beyond the age at which their index case had either died or were bedridden.

Among the 8 pre-symptomatic LI subjects at the time of treatment, 4 subjects were within the range of gross motor function observed in a healthy cohort of children from Palisano (1997) of similar chronological age throughout their follow-up and were consistent with the physiological progressive acquisition of new motor skills (Palisano, 1997).

The 4 pre-symptomatic LI subjects who showed GMFM scores post-GT below scores from the healthy cohort at a similar age all had abnormal neurological examination findings at Baseline.

Early Juvenile (EJ) MLD

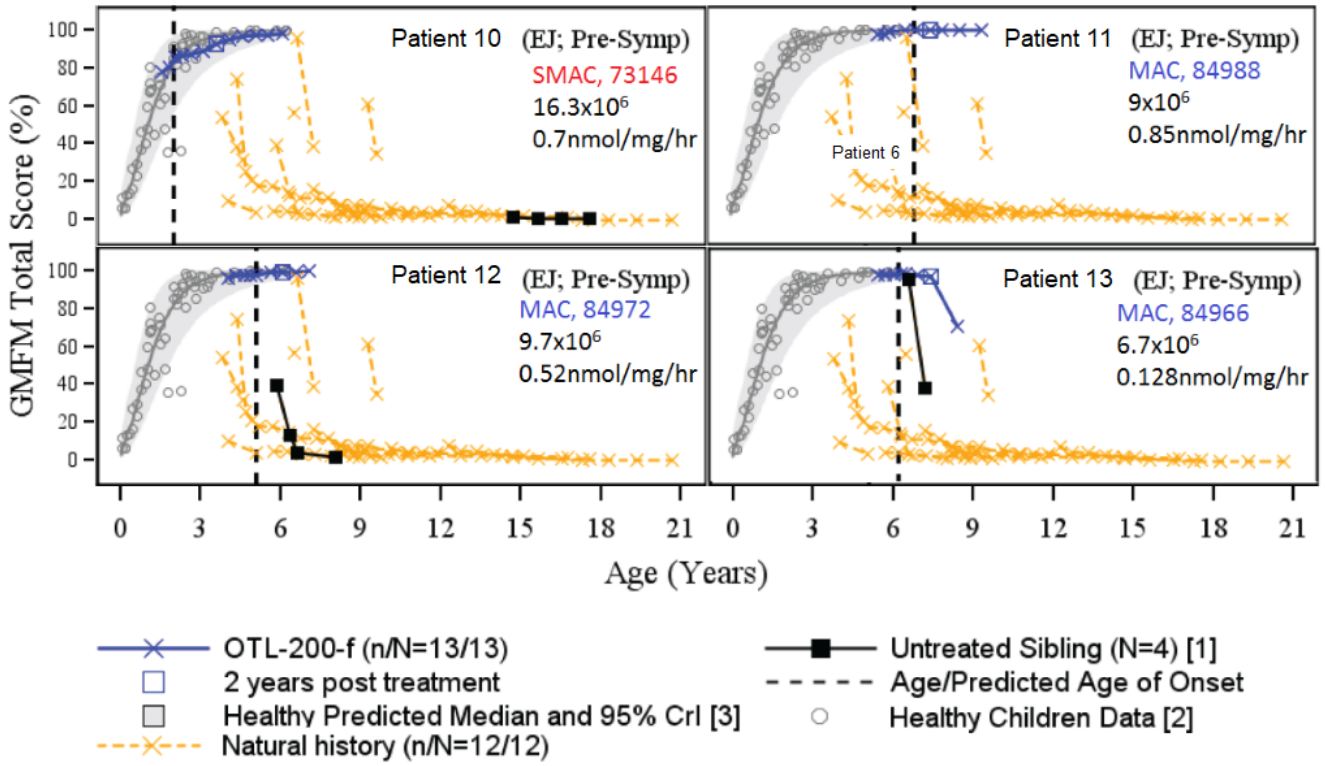
For the overall *EJ MLD* subgroup the mean total GMFM score at 2 years was 76.5 for the Libmeldy treatment group compared to 36.6 points for the historical (Difference 39.8 points 95%CI 9.6; 80.1, p=0.026).

pre-symptomatic EJ MLD

For the pre-symptomatic EJ MLD, the adjusted LS mean GMFM total score at year 2 post treatment was 96.7%. Difference from the NHx group was 52.4% (95% CI 25.1; 79.6, p=0.008) at year 2.

Individual panel plots are presented in Figure 14.

Figure 14: Pre-Symptomatic Early Juvenile subgroup: Panel Plot of Gross Motor Function Measure Total Score (%) Over Time Compared to TIGET NHx Data*



[1] Untreated sibling data is a subset of NHx data.
[2] If there are 2 reference lines for "Age/Predicted Age of Onset," this reflects the range given in the eCRF.

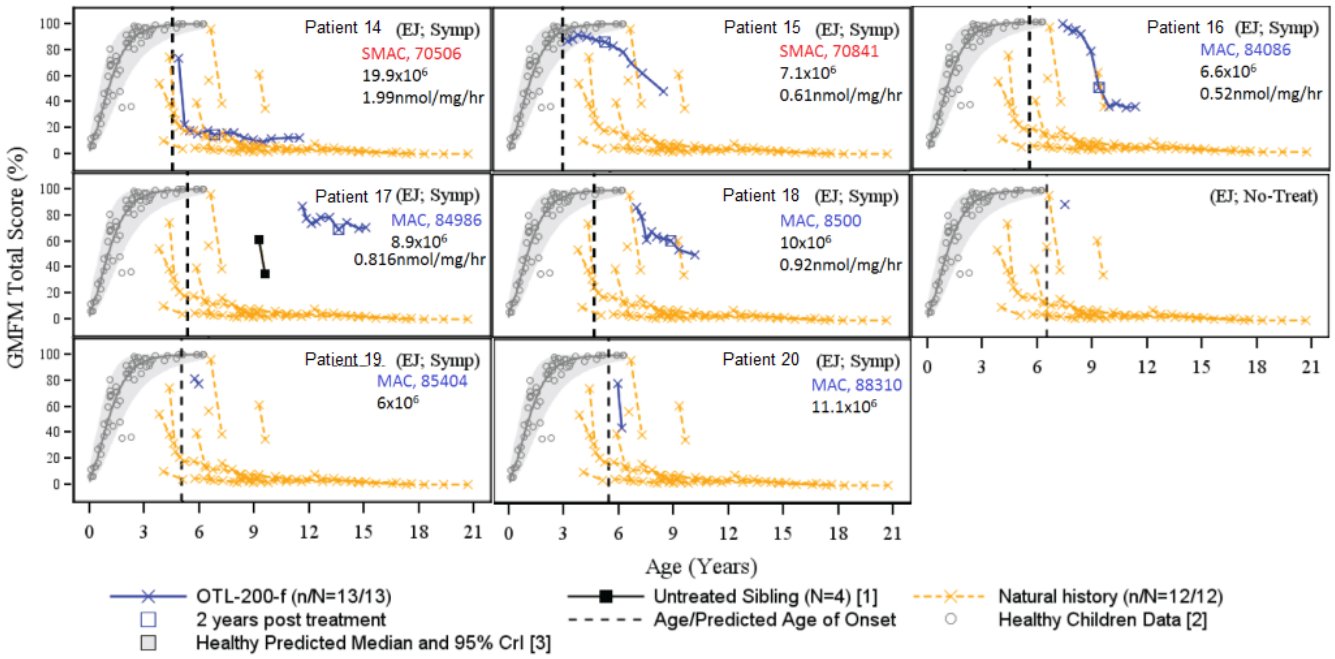
[3] Healthy children data from Dr. Palisano and colleagues, who provided access to the anonymous age and GMFM-88 data on 60 subjects in the "No CP" group as reported in (Palisano, 1997).
The triangle included in all panels represent the baseline value measured for one subject who was not treated due to rapid disease progression.
*figure adapted by Assessor to include:
- Dose (CD 34+ cells/kg)
- ARSA CSF activity levels indicated measured 2 years post treatment (primary endpoint marked by applicant).
- busulfan conditioning: SMAC and MAC treatment indicated with corresponding AUCs
Note: As of Protocol Amendment 11, the drug product name has been changed from GSK2696274 to Libmeldy-f.

Symptomatic EJ MLD

For the symptomatic EJ MLD, the adjusted LS mean GMFM total score at year 2 post treatment was 60.7%. Difference from the NHx group was 28.7% (95% CI -14.1; 71.5, p=0.35) at year 2. At Year 3 there was a difference in treatment effect of 43.9% (59.8% vs. 15.9%; p=0.054. At Year 4 and Year 5 this difference was confirmed (42.9% for both: 53.6% vs. 10.7% [p=0.054] and 50.3% vs. 7.4% [p=0-107], respectively).

Individual panel plots are presented graphically in Figure 15.

Figure 15: Early-Symptomatic Early Juvenile Subgroup: Panel Plot of Gross Motor Function Measure Total Score (%) Over Time Compared to TIGET NHx Data*



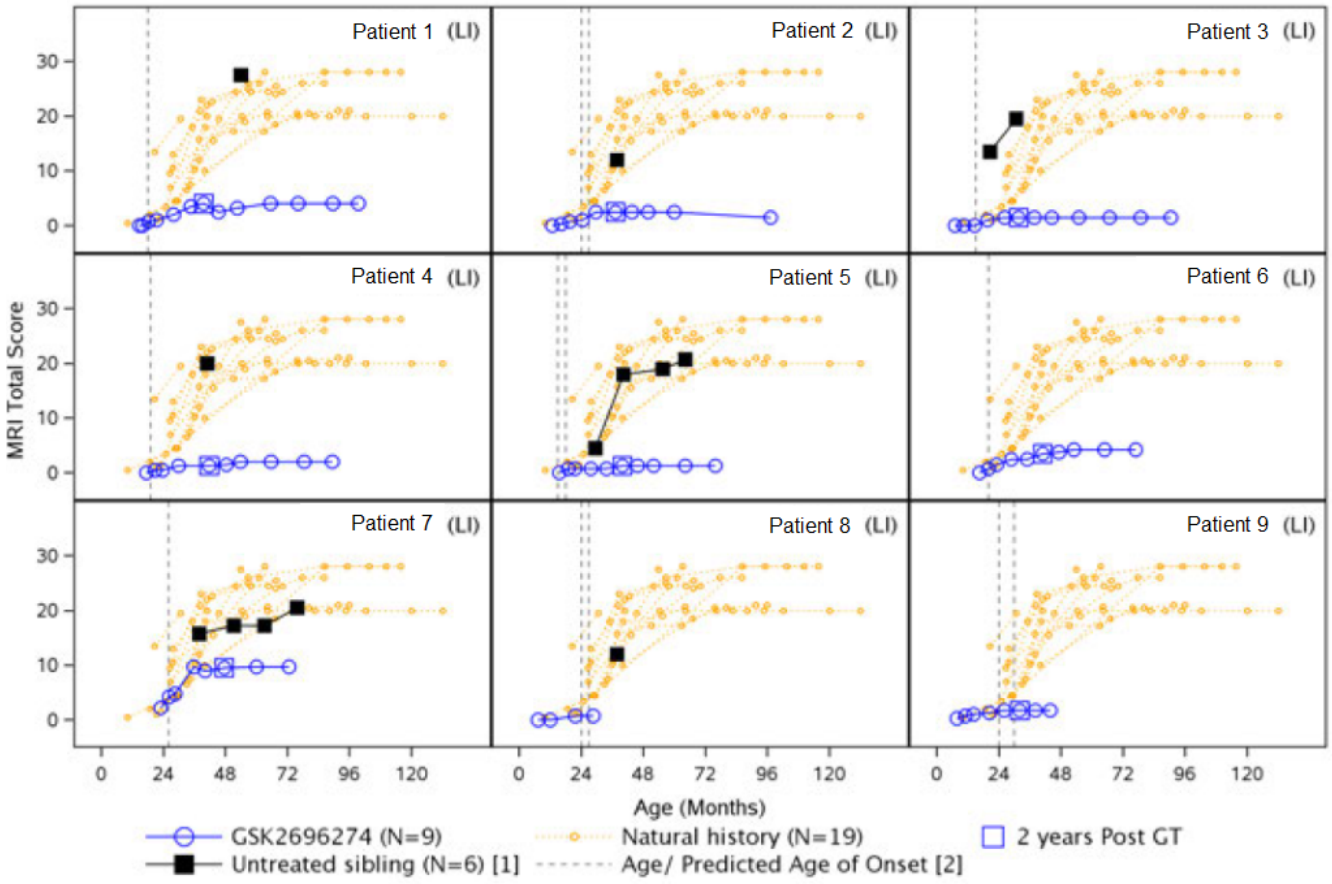
[1] Untreated sibling data is a subset of NHx data.
[2] If there are 2 reference lines for "Age/Predicted Age of Onset," this reflects the range given in the eCRF.
[3] Healthy children data from Dr. Palisano and colleagues, who provided access to the anonymous age and GMFM-88 data on 60 subjects in the "No CP" group as reported in (Palisano, 1997).
The triangle included in all panels represent the baseline value measured for one subject who was not treated due to rapid disease progression.
*figure adapted by Assessor to include:
- Dose (CD 34+ cells/kg)
- ARSA CSF activity levels indicated measured 2 years post treatment (primary endpoint marked by applicant).
- busulfan conditioning: SMAC and MAC treatment indicated with corresponding AUCs
Note: As of Protocol Amendment 11, the drug product name has been changed from GSK2696274 to Libmeldy-f.

BRAIN MRI_

Late Infantile MLD (LI MLD)

Stabilisation of brain MRI total score was seen between Year 2 and Year 3 post-GT in all LI subjects except for two subjects (Patient 4 and 6), who stabilised later at Year 3 post-GT. The MRI score mean differences between Libmeldy treated LI subjects and NHx subject of -11.8 ($p<0.001$). (see Figure 16 for individual panel plots).

Figure 16: Late Infantile Subgroup: Panel Plot of Brain Magnetic Resonance Imaging Total Score Over Time Compared to TIGET NHx Data



[1] Untreated sibling data is a subset of NHx data.
[2] If there are 2 reference lines for "Age/Predicted Age on Onset," this reflects the range given in the eCRF.
Note: As of Protocol Amendment 11, the drug product name has been changed from GSK2696274 to Libmeldy-f. Source: Listing 2.44

All LI subjects pre-symptomatic at the time of treatment (8/9) stabilised at a significantly lower MRI total score (≤4.25) than untreated NHx subjects at a comparable chronological age of 53 months, supporting a relevant treatment effect of Libmeldy-f on the typical brain involvement (demyelination and atrophy) observed in MLD.

The outcomes for nerve conduct velocity, parent reported outcomes and neuropsychological tests were consistent witht the GMFM findings.

Early Juvenile (EJ) MLD

Difference between the Libmeldy treated EJ MLD and NHx was -4.1 points, p=0.12, on brain MRI. For the *pre-symptomatic EJ MLD*, the differences in the adjusted LS mean MRI total scores between the Libmeldy treated pre-symptomatic EJ subjects and NHx subjects was 10.7 (95%CI 7.0, 14.4; p<0.001). For the *Symptomatic EJ MLD*, the difference in LS mean MRI total score between the Libmeldy treated symptomatic subjects and NHX was 5.8 (95% CI -4.0, 15.5; P=0.21).

SURVIVAL

Late Infantile MLD (LI MLD)

At the latest observation visit covered by this study report, the mean follow-up for all LI subjects treated is 5.4 years (range 2.98 to 7.51 years) and all subjects remain alive (overall survival 100%). Because no deaths occurred in the LI subgroup treated with Libmeldy-f, no median survival time is available.

Early Juvenile (EJ) MLD

Two of the 11 (18%) subjects in the EJ subgroup treated with Libmeldy-f had died due to disease progression (parents elected not to continue full supportive care), and 3 of the 12 (21%) TIGET NHx Study participants had died at the time of the interim analysis. However, none of the *pre-symptomatic EJ MLD* patients treated died. Two of the 7 subjects in the *symptomatic EJ MLD* subgroup treated with Libmeldy-f died due to disease progression (parents elected not to continue full supportive care).

OTHER SECONDARY OUTCOMES

Late Infantile MLD (LI MLD)

The outcomes for the GMFM-MLD, nerve conduct velocity, neuropsychological test, parent reported outcome were consistent with the effects seen for the GMFM.

Early Juvenile (EJ) MLD

For the overall *EJ MLD* population the outcomes for the GMFC-MLD, nerve conduct velocity, neuropsychological test, parent reported outcome were generally consistent with the effects seen in the GMFM. *Pre-symptomatic EJ MLD* subjects had scores for GMFC-MLD, nerve conduct velocity, neuropsychological test within ranges reported for healthy subjects.

Symptomatic EJ MLD. Throughout the extended follow-up period, 6/8 Symptomatic EJ subjects remained stable and above the threshold for sever cognitive impairment (IQ≥55) with a statistically significant difference from the NHx controls at Year 2 (IQ 88.9 vs. 31.9 in NHx controls; treatment difference 61.1, p=0.029), Year 3 (89.4 vs. 17.6; treatment difference 71.8, p=0.013), Year 4 (81.9 vs. 15.2; treatment difference 66.8, p=0.026), but not at Year 5 (48.2 vs. 9.8) probably due to the small numbers.While deterioration is observed in all symptomatic EJ subjects on motor function, the cognitive function appears to be preserved in subjects with an IQ>85 and a GMFC-MLD ≤1. On both group and individual levels an effect on cognitive function is seen as the IQ is comparable or higher compared to healthy children of the same age.

Minimal Clinically Important Difference (MCID)

The applicant conducted an exploratory analysis to define the minimal clinical important difference. The minimal clinical important difference was defined by the applicant as the GMFM total score decrease causing a change in one GMFC-MLD level. The applicant plotted the GMFC-MLD against the GMFM total score. There is a

linear relationship between the decrease in the mean GMFM total score and the increase of GMFC-MLD levels and specific percentages of the GMFM total score correspond to specific levels of GMFC-MLD. A 15% change in GMFM corresponds to a 1 category change in GMFC-MLD level. The treatment effects of Libmeldy on GMFM (%) total scores in surviving early symptomatic EJ patients at Year 2, Year 3, Year 4 and Year 5 post-treatment ranged between 28.7% and 42.9%, and therefore 2-3 times the MCID identified in the exploratory analysis. The results were consistent when only the 5 early symptomatic EJ subjects meeting the current proposed cut of points for treatment (IQ score ≥85 or GMFC-MLD<1) would have been included. In more practical terms, while at the same chronological age untreated NHx controls had lost all locomotion function and head and trunk control indicated by a GMFC-MLD level of 6, the early symptomatic EJ subjects had maintained part of their motor function (two subjects, both at level 3) or even were able to walk independently for short distance (one subject).

QoL-related composite endpoint "Severe Motor Impairment Free Survival (sMFS)

The applicant reported results of analysis on the QoL-related composite endpoint "Severe Motor Impairment Free Survival (sMFS)" defined as the interval from birth to the earliest point of loss of locomotion and sitting without support (GMFC-MLD Level 5 or higher) or death. The individuated corresponding level of GMFC-MLD at which QoL is severely impacted was 5. A survival analysis on early symptomatic EJ variant showed that 3/8 subjects (37.5%) experienced sever motor impairment (one subject) or died (two subjects) corresponding to a 62.5% estimated probability of remaining event-free (maintained locomotive and/or sitting abilities, GMFC ≤4) at a chronological age of 8 years compared with a 36.0% estimated probability for untreated EJ subjects. All but one of the surviving EJ subjects (5/6, 83%) treated at an early-symptomatic stage remained event-free throughout follow-up compared with none in the untreated EJ group (unstratified log-rank test for all treated early symptomatic EJ subjects vs. untreated subjects p=0.031). Even when the sMFS was evaluated as time to event from disease onset, the results were consistent and by 4 years post-disease onset, an estimated 62.5% of treated early symptomatic EJ subjects survived and maintained locomotion and ability to sit without support compared with 26.3% of untreated EJ subjects.

As of the new data cut of December 2010, no additional ES EJ patients died or met the criteria for severe motor impairment (GMFC ≥5). Five of 6 (83%) surviving ES EJ MLD patients have remained event-free throughout the extended follow-up compared with none in the untreated EJ group (unstratified log-rank test for all treated ES EJ subjects vs. untreated subjects for age at sMFS p=0.033, log rank for time from disease onset to sMFS p=0.063).

Ancillary analyses

N/A. The main study is presented based on the following subgroups: the LI MLD, pre-symptomatic EJ MLD subjects and symptomatic EJ MLD subjects.

Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy, as well as the benefit risk assessment (see later sections).

Table 10: Summary of efficacy for trial 201222

Title: A Phase I/II Clinical Trial of Haematopoietic Stem Cell Gene Therapy for the Treatment of Metachromatic Leukodystrophy			
Study identifier	Study 201222 EudraCT number 2009-017349-77 ClinicalTrials.gov: NCT01560182		
Design	Open-label, non-randomised, prospective, single-centre, pre-symptomatic late infantile metachromatic leukodystrophy (MLD), pre- or early-symptomatic early juvenile MLD		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		2 years 35 days 8 years
Hypothesis	Superiority versus natural history cohort (or untreated sibling when available)		
Treatments groups	LI- MLD		Libmeldy-f. mean duration follow up = 5.4 yrs, n=9 included (incl. 1 symptomatic)
	EJ- MLD		Libmeldy-f. mean duration follow up= 3.5 yrs , n=11 included (4 Pre-Symptomatic, 7 Symptomatic)
	LI-MLD TIGET NHx		Untreated. mean duration follow up= 6.8 yrs, n=19 included
	EJ-MLD TIGET NHx		Untreated. mean duration follow up = 6.8 yrs, n=12 included (8 Pre-Symptomatic, 10 Symptomatic)
Endpoints and definitions	Co-Primary endpoint	ARSA activity	a significant increase of residual ARSA activity as compared to subjects' pre-treatment values at year 2 post treatment. ARSA activity measured in PBMC, BM and CSF.
	Co-Primary endpoint	GMFM	reduction in the progression of the clinical motor impairment in treated subjects at 2 years post treatment compared to the progression measured in untreated MLD subjects within the TIGET – NHx Study.
	Secondary endpoint	% LV	sustained engraftment of the transduced cells, an essential pre-requisite for achieving clinical benefit.
	Secondary endpoint	Brain MRI	Evaluation of the efficacy of the procedure in reducing the progression of demyelination in the central and PNS (as well as atrophy in the CNS) at 2 years post treatment in comparison with that documented in TIGET NHx Study participants.
	Secondary endpoint	Neuro-psychological tests	Evaluation of the efficacy of the procedure in reducing the progression of the cognitive impairment at 2 years post treatment.

	Secondary endpoint	Survival	Evaluation of the survival between subjects treated with GT and NHx data.
Database lock	30-Mar-2018		
Results and Analysis			
Analysis description	Primary Analysis		
Analysis population and time point description	Intent to treat Year 2		

Descriptive statistics and estimate variability				
	Treatment group	*LI-MLD*	*EJ-MLD*	
			Pre-Symp.	*Symp.*
	Number of subjects	9	11	
			4	7
	ARSA activity	Mean 0.852 (95%CI: 0.581; 1.251)	Mean 0.64 nmol/mg/hr (95%CI 0.37; 1.13)	
	GMFM (mean)	72.5%	76.5%	
			96.7%	60.7%
	% LV transduced cells in BM	Mean 56.07 (95% CI 39.94; 78.71)	Mean 47.11 (95% CI: 34.06; 65.16)	
	VCN/Cells in PBMC	Mean 0.6744 95% CI: 0.3068;1.482	Mean 0.5008 (95% CI: 0.3106; 0.8075)	
	Brain MRI	2.1	9.1	
			5.0	13.5
	Survival (subjects with event, died)	0	2	
			0	2

Effect estimate per comparison	*Late Infantile*		
	Co-Primary Endpoint	Comparison groups	LI-MLD TIGET NHx (n=9) vs LI-MLD Libmeldy (n=8)
	GMFM	Mean; difference (δ)	7.4 vs 72.5; δ=65.1
		95% CI for difference	41.6, 88.6
		P-value	<0.001
	Secondary endpoint	Comparison groups	LI-MLD (n=15) vs LI-MLD Libmeldy (n=8)
	Brain MRI	Mean; difference (δ)	13.9 vs 2.1; δ = -11.8
		95% CI for Difference	(-15.4, -8.1)
		P-value	<0.001

	Secondary endpoint *Survival*	Comparison groups	LI-MLD TIGET NHx vs LI-MLD Libmeldy-f
		Median estimated age to death (months)	134.9 vs unknown
		95% CI	68.4;160.8
		Log-Rank P-value	0.062
	Early Juvenile (full population)		
	Co-Primary Endpoint *GMFM*	Comparison groups	EJ-MLD TIGET NHx (n=11) vs EJ-Libmeldy (n=9)
		Mean; difference (δ)	36.3 vs 76.5; δ=39.8
		95% CI for difference	9.6;70.1
		P-value	0.026
	Secondary endpoint *Brain MRI*	Comparison groups	EJ-MLD TIGET NHx (n=11) vs EJ-MLD Libmeldy (n=9)
		Mean; difference (δ)	13.2 vs 9.1; Difference = -4.1
		95% CI for Difference	(-9.6; 1.3)
		P-value	0.123
	Secondary endpoint *Survival*	Comparison groups	EJ-MLD TIGET NHx vs EJ-MLD Libmeldy-f treatment
		Estimated HR	1.85
		95% CI	0.26; 13.30
		Log-Rank P-value	0.537
	Pre-Symptomatic EJ-MLD population		
	Co-Primary Endpoint *GMFM*	Comparison groups	Pre-symptomatic EJ-MLD Libmeldy vs EJ-MLD NHx
		Mean; difference (δ)	96.7 vs 44.3%; δ=52.4%
		95% CI for difference	25.1; 79.6
		P-value	0.008
	Secondary endpoint *Brain MRI*	Comparison groups	Pre-symptomatic EJ-MLD Libmeldy vs EJ-MLD NHx
		difference (δ)	10.7
		95% CI for difference	7.0,14.4
		P-value	<0.001
	Symptomatic EJ-ML-population		
	Co-Primary Endpoint *GMFM*	Comparison groups	symptomatic EJ-MLD Libmeldy vs EJ-MLD NHx
		Mean; difference (δ)	60.7% vs 32%; δ=28.7%
		95% CI for difference	-14.1; 71.5
		P-value	0.35
	Secondary endpoint	Comparison groups	symptomatic EJ-MLD Libmeldy vs EJ-MLD NHx

	Brain MRI	difference (δ)	5.8
		95% CI for difference	-4.0, 15.5
		P-value	0.21
Notes	Abbreviations: ARSA = Arylsulfatase A, BM = Bone Marrow, CI= Confidence Interval, CSF = cerebral Spinal Fluid, EJ = early juvenile, GMFM= Gross Motor Function Measure, GMFC = Gross Motor Function Classification, LI = Late Infantile, LV = Lentiviral Vector , MLD = Metachromatic Leukodystrophy, MRI= Magnetic Resonance Imaging, NHx = Natural History, Libmeldy, PBMC= Peripheral Blood Mononuclear Cells, SD = Standard Deviation, TIGET = Telethon Institute for Gene Therapy		
Analysis description	Comparison analysis against TIGET NHx		
	The analysis method was MMRM adjusted for Visit, Base, Base*Visit, Disease Subtype and Disease Subtype*Visit, and Toeplitz correlation matrix was used. The analysis method was an ANCOVA for each disease subtype and analysis visit was adjusted for treatment, age and an age by treatment interaction term. A negative difference indicates a better outcome in the GSK2696274 arm. P-value for two-sided 5% hypothesis test with null hypothesis of no difference. No distinction is made for symptomatic state. However, this appears to affect the outcome. Particularly between early symptomatic (i.e. IQ ≥ 85 and GMFC-MLD≤ 1		

Clinical studies in special populations

Not Applicable

Supportive studies

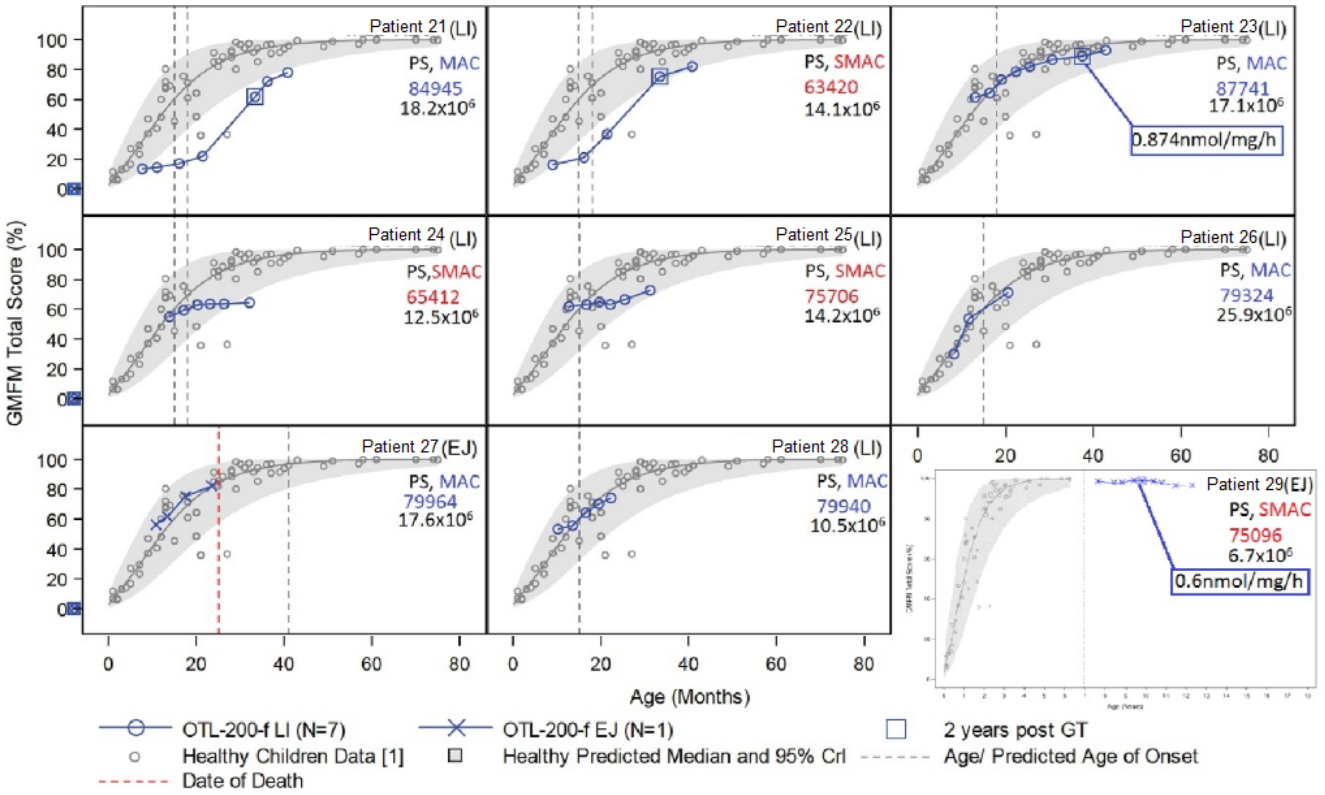
Results. Seven pre-symptomatic LI subjects and 2 pre-symptomatic EJ subjects were treated in EAP (expanded access programmes). All tested patients showed a high percentage of %LV+ cells (geometric mean: 92.71%, range: 85.7% to 98.4%; n=5), 28 days after treatment with Libmeldy-f. This engraftment was sustained 12 months post-treatment (geometric mean: 92.47%, range: 80.1% to 100.0%; n=5).

At Year 1, a 64-fold increase (95% CI: 19-fold, 214-fold; n=8) in ARSA activity in PBMCs was observed compared with baseline levels (Table 14.2.2.2). The mean value at Year 1 (1830.1 nmol/mg/h) was 9.24-fold higher than the upper limit of the ARSA activity normal range (198.02 nmol/mg/h). At time points beyond Year 1, confidence intervals are currently large due to the limited number of patients contributing data; however, the data to date shows stabilisation at levels similar to the mean observed at Year 1 (Year 2, 2164.2 nmol/mg/h, n=3; Year 2.5, 2199.1 nmol/mg/h, n=3).

Consistent with ARSA activity in PBMCs, mean ARSA activity levels in total BM mononuclear cells also increased substantially as early as 3 months after treatment. By 3 months after treatment with Libmeldy-f, mean ARSA activity levels in total BM MNC were 37.6-fold (95% CI: 24.22-fold, 58.40-fold; n=5) higher than baseline. Throughout the course of follow up, mean ARSA levels in total BM mononuclear cells remained at least 55-fold higher than baseline.

Individual panel plot for GMFM are presented in Figure 17.

Figure 17: Panel Plot of GMFM Total Score (%) Profiles



NOTE: Healthy children data were from Palisano and colleagues, who provided access to the anonymous age and GMFM88 data on 60 subjects in the "no CP" group as reported in Palisano, 1997.
Two reference lines for 'Predicted Age of Onset' reflect the range of ages provided in the eCRF.
CP=cerebral palsy; CrI=credible interval; eCRF=electronic case report form; EJ=early juvenile; GMFM=gross motor function measure; GT=gene therapy; LI=late infantile

The results of the Brain MRI severity scores, nerve conduction velocity (NCV), Neuropsycholoical tests, Gross Motor Function Classification in MLD (GMFC-MLD), nine-hole peg test and lansky performance were consistent with the outcomes reported for the in total GMFM score.

Survival. In studies HE 205029 and CUP 206258, 7 of the 8 patients (87.5%) enrolled in the EAPs were alive as of the data cut. One patient (Patient 27) died due to ischemic cerebral infarction, which is considered unrelated to Libmeldy-f (se Clinical AR section 4.4.2 deaths for narrative).

Study 205756 is an open-label, single-arm study in pre-symptomatic LI MLD or EJ MLD subjects.

This study was performed with crypopreserved formulation and submitted in order to substantiate that the clinical efficacy between the fresh and cryopreserved formulation is similar. Based on the previous experience only pre-symptomatic subjects were included.

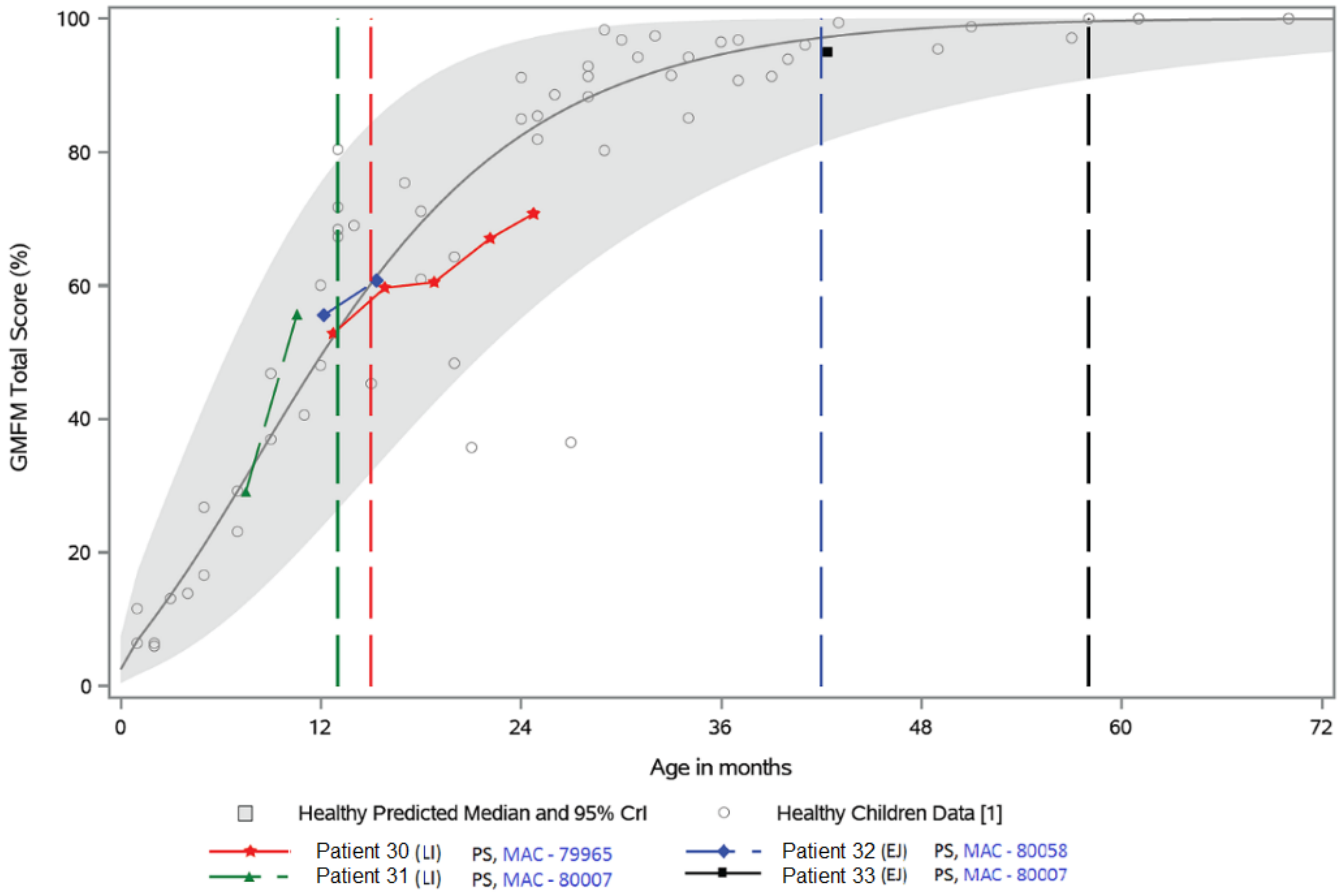
Subjects treated with Libmeldy-c showed comparable levels of in the proportion of LV-Positive Colony-Forming Cells in BM: At Month 1 post-treatment, all subjects tested (n=3) showed high levels of genetically modified cells in BM, with a range between 70.83% LV+ cells and 80.83% LV+ cells. At Month 3, data were available for two Subjects (Patient 31 and 32), showing 89.39% LV+ cells and 80% LV+ cells, respectively, consistent with the level observed at Month 1 for one Subject (Patient 32). Post-treatment, all subjects with available data had ARSA values within or above the normal range (normal range=30.56-198.02 nmol/mg/h)

at Month 1 and above the normal range at Month 2. Levels above the normal range were maintained at Month 3, Month 6, and Year 1 for the subjects with available data.

At Baseline, ARSA activity levels in CSF were undetectable in all 4 subjects. After administration of Libmeldy-c, ARSA activity levels were detectable and within the normal range at Month 3 in all three subjects with available data (three Subjects- Patients 30,31,32).

Preliminary data on *GMFM* total score showed that gross motor function for all 4 subjects was within the range of gross motor function observed in a healthy cohort of children from (Palisano, 1997) of similar chronological age (grey shade) (Figure 18).

Figure 18: Plot GMFM total Score (%)



*Secondary outcomes w*ere consistent with the findings from the main study.

Extrapolation plan from LI MLD and EJ MLD to LJ MLD

The applicant originally proposed an extrapolation plan to include treatment of LJ MLD patients based on

i) the positive benefit/risk profile shown in the source early-onset MLD population (particularly in symptomatic domains which are most relevant for late-onset MLD patients),

ii) the arbitrary age separation between the EJ and LJ variants,

iii) the common disease pathophysiology across MLD variants,

iv) the shared mechanism of action of Libmeldy to address the underlying pathophysiological mechanisms of the disease, along with

v) the larger window of opportunity in late-onset MLD and

vi) the post-approval clinical activities proposed to address the gaps in knowledge.

Full extrapolation from LI MLD and EJ MLD to LJ MLD was not agreed. While the distinction between LI MLD /EJ MLD and LJ is arbitrary, the disease pathology is similar across these disease subtypes, and the mechanism of action of OLT-200 is similar across the disease subtypes it may be rational to extrapolate the efficacy of treatment as seen in the subjects with early onset of disease (LI MLD /EJ MLD) to the older paediatric population (LJ MLD). However, it should be noted that for subjects with LJ MLD the disease severity and the life expectancy is different compared with subjects with LI MLD and EJ MLD. The LJ-MLD variant is closer to the adult MLD than to the EJ and LI variant of the disease. As a consequence, the benefit and the risks of treatment should be weighed differently. Furthermore, LJ subjects are identified by symptoms and the symptomatic status of the patient is vital for the treatment success, data is required to conclude on prognostic values for treatment success in LJ MLD.

Therefore, the applicant has now removed LJ MLD from the proposed indication. The applicant intents to submit data from an open label non-randomised study to extend the indication to LJ MLD subjects.

2.7.1. Discussion on clinical efficacy

The applicant originally proposed the following indication:

"*Treatment of metachromatic leukodystrophy (MLD) in patients from birth to before 17 years and in older patients for whom disease onset occurred before 17 years.*

Treatment with Libmeldy should be performed before the disease enters its rapidly progressive phase."

MLD is a rare autosomal recessive inherited lysosomal storage disorder caused by mutations in the ARSA gene that results in deficiency of its corresponding enzyme. The disease spectrum can present in different clinical forms, but there is no universally accepted classification system for MLD phenotypes and at least 3 clinical forms of the disease are commonly described (late infantile [LI], juvenile, and adult MLD). The juvenile forms are further stratified into early (EJ) and late juvenile (LJ).

The clinical development programme supporting the requested indication is based on 2 clinical studies and 3 Expanded Access Programmes (EAPs), with total of 33 patients (18 LI and 15 EJ; 24 pre-symptomatic and 9 early-symptomatic).

Design and conduct of clinical studies

In support of the claimed indication, treatment of MLD in patients before the age of 17 years, efficacy results have been submitted of 33 LI MLD and EJ MLD patients who were treated with Libmeldy in a total of 5 clinical studies with a similar design.

The studies concerned non-randomised, open-label, prospective, comparative (natural history cohort control), single centre studies. Each study had a screening phase, baseline phase, treatment phase and follow-up phase.

No specific dose-response studies were conducted, and the dose range to be used in clinical studies was based on experience with other autologous-ex-vivo gene therapy programmes, which is acceptable based on EMA Guidelines [EMA/CAT/GTWP/671639/2008 and EMA/CAT/GTWP/671639/2008/Rev. 1].

PD parameters, which consists of engraftment of LV transfected cells and ARSA activity levels were measured in all studies.

Additional analyses seem to confirm that cell dose levels as DP quality attribute can have an impact on early cell engraftment and peripheral PD parameters (ARSA activity in PBMCs) but not on haematological reconstitution and clinical efficacy outcomes. Therefore, it was not possible to identify a clear cut-off of cell dose predicting efficacy in terms of motor and cognitive function. For this reason, the dose range recommendation proposed in section 4.2 on the SmPC that are based on the experience derived from the field of allogenic and autologous HSCT and the body of evidence on Libmeldy is acceptable.

The design of the main study, e.g. single arm with natural history cohort, was agreed by the SAWP given the severity and progressive nature of the disease, the absence of curative treatment, the possibility of Libmeldy to be curative treatment and the limited size of the target population. Despite the well-known limitations related to the use of a NHx control group for registrational studies, even in rare diseases, it is acknowledged that the applicant's efforts allowed to improve the matching strategies based on age and disease variant and, hence possibly by disease stage, by implementing both retrospective and prospective data collection, reducing the variability in operator assessment (i.e. the same staff administered tools/assessment to both NHx and treated subjects), and using the same methodology, instrumentation, and assessments.

All subjects enrolled in the registrational Study 201222 and in the NHx cohort had an MLD diagnosis based on molecular, biochemical, and clinical parameters and pseudodeficiencies were excluded based on the presence of two disease-causing mutations and the presence of an affected sibling.

The use of a natural history cohort consisting of 31 untreated LI MLD and EJ MLD subjects as well as data from an untreated sibling with MLD to serve as a control was also agreed. MLD is very heterogeneous and the use of these comparators help to contextualise the results.

The studies included pre-symptomatic and early symptomatic LI MLD and EJ MLD subjects. An extrapolation of the outcomes from the clinical studies in LI MLD and EJ MLD subjects to LJ MLD population was proposed based on the similarity in disease pathology and the mechanism of action of Libmeldy.

All LI MLD and some pre-symptomatic EJ MLD subjects were identified after an older sibling developed symptoms and was diagnosed with MLD, prompting family testing. LI MLD was defined as patients with an age at onset in the older sibling ≤30 months, and/or 2 null (0) mutant ARSA alleles, and/or peripheral neuropathy at electroneurographic study. EJ MLD was defined as age of onset in the subject or effected sibling between 30 months and 7th birthday, and/or 1 null (0) and 1 residual (R) mutant ARSA alleles, and/or peripheral neuropathy at electroneurographic. These correspond with the general distinction for the MLD variants.

Pre-symptomatic status was defined as subjects without disease related symptoms or with no abnormalities observed in the electroneurographic study and brain MRI. Early symptomatic status was only defined for the EJ-MLD population. This was initially within 6 months after the first reported symptom, and later as subjects with an intelligence quotient ≥70 and the ability to walk independently for ≥10 steps. The definition of early

symptomatic is not a general accepted term, but a criterion defined by the applicant. The change to include a threshold for walking and intelligence quotient can be agreed, as this takes into account the heterogeneity of the MLD and is independent of the progression rate.

In addition to the definition of early symptomatic, the applicant introduced several changes to the clinical programme. These concern several modifications to the manufacturing process, switch in formulation from fresh to cryopreserved, the possibility to collect starting material from peripheral blood by apheresis instead of bone marrow puncture, and a change in the myeloablative condition regimen with busulfan. Patients received initially a SMAC regimen (4800 µg*h/L) which was subsequently modified to a MAC regimen (67,200 µg*h/L) with the aim to reduce the variability of transduced cell engraftment.

All studies had the same co-primary endpoints consisting of an improvement in the total GMFM score and an increase in ARSA activity in PBMCs, both assessed 2 years post treatment. The treatment was deemed a success when a delay in progression of 10% of the total GMFM score and a 2x standard deviation increase in ARSA activity levels was recorded.

Secondary outcomes include brain MRI, Gross Motor Function Classification in MLD (GMFC-MLD), ARSA activity in CSF, neuropsychological tests, survival etc. Overall, the chosen primary and secondary endpoints allow assessment of neuronal development (GMFM, neuropsychological testing) and disease progression (brain MRI, NVC, GMFC-MLD, GMFM and neuropsychological testing). While the combination of an efficacy parameter (GMFM score) and a pharmacodynamic parameter (ARSA activity) at a certain point in time can be accepted as a primary endpoint, for a proper appreciation of the effect of treatment and given the limitations to the patient population the full data pack will need to be taken into account. Notably, there is an overlap in the different endpoints, e.g. GMFC-MLD and GMFM both assess motor function. As regard to ARSA activity, it is acknowledged that peripheral ARSA activity measured in PBMC better reflects the mechanism of action of Libmeldy as modified cells infused systematically that should subsequently migrate into the CNS and PNS after crossing the blood-brain barrier. For efficacy ARSA activity in CNS and PNS is needed and thus ARSA CSF levels are of specific interest. The applicant's hypothesis that ARSA activity in CSF may represent only a downstream expression of enzymatic reconstitution in the brain could be biologically plausible, however, this has not been proven with specific studies. Therefore, although ARSA activity in CNS does not take into account the enzymatic activity in PNS, it should represent one of the direct markers of Libmeldy mechanism of action and efficacy at central level. Indeed, focus of the assessment will be on the ARSA activity in the CSF, the GMFM assessment (primary endpoint), brain MRI and neuropsychological testing as this allows assessment on molecular level, morphological level, motor development and cognitive function.

The effect of the change in formulation of Libmeldy on the efficacy, i.e. Libmeldy-f vs Libmeldy-c, is assessed by the applicant in a separate clinical study. The data is compared to the pivotal study. This study includes 4 subjects, 2 EJ MLD and 2 LI MLD. The follow up time ranged from 1 month to 1 year. This study also had a similar design as the pivotal study with the same Co-primary endpoint.

Efficacy data and additional analyses

PK-PD analysis. Engraftment of bone marrow derived colonies harbouring the lentiviral genome was successful in all subjects. Levels increased within 28 days 59.1% and was 54.8% (CI 95% 44.1; 68.2%, [n=23]) at Year 1 post treatment. At Year 5, that proportion was 45.0% (CI95% 24.1%; 84.2% [n=6]). The VCN in the PBMCs increased to approximately 1 VCN/cell for the LI MLD and approximately 0.25 VCN/cell for the EJ MLD variant.

VCN values in total PBMCs had a geometric mean of 0.19 copies/cell (range 0.03 to 0.68; n=29) at Day 28 post gene therapy. In both the LI and EJ subgroups, the VCN in total PBMCs increased over time and remained relatively stable from Year 1 post-treatment throughout the course of follow-up. The engraftment could be affected by the source material used. Originally data from 4 mobilised peripheral blood apheresis compared to 33 bone marrow derived batched were available. The applicant provided 6 additional batches from mobilised peripheral blood apheresis. Although some differences are observed in cell composition that can be attributed to the difference in starting material, there are no indications that this results in differences in clinical outcome. The VCN and ARSA activity in transduced cells was on average lower in mPB-derived batches. Results were, however, within the range observed for BM-derived batches.

The applicant was requested in a Scientific Advice to link ARSA CSF levels to clinical outcome. Therefore, an analysis to determine whether a correlation between the ARSA CSF levels and GMFM, GMFC-MLD, DQ, MRI exist was performed. No correlation was found between ARSA CSF level and any of the clinical outcome measures examined (motor function, cognition or MRI total scores regardless the MLD variant and timepoints (2 Years and 3 Years post treatment).The applicant stated that CSF is a surrogate compartment of brain and hypothesised that levels of ARSA activity in CSF may represent a downstream expression of enzymatic reconstitution in the brain, reflecting the diffusion of a limited fraction of the supraphysiological enzymatic levels produced and secreted to the extracellular matrix for cross-correction. Furthermore, levels of enzyme activity reported might be influenced by the protein content in the CSF sample. These hypotheses will be further elucidated in a post marketing setting to allow evaluation of ARSA CSF activity as predicting factor for treatment success or explanation of failure, if possible.

Pivotal study:

The study included 9 LI subjects and 11 EJ subjects. The number of subjects included in the pivotal study is limited. All LI subjects were pre-symptomatic at time of screening. One subject (Patient 7) became symptomatic prior to treatment. In the EJ group, 4 subjects were pre-symptomatic and 7 subjects were early symptomatic at the screening phase. This did not change during the treatment phase.

The follow up is 8 years, and at time of data cut off, the mean follow up was 5 years in the LI subjects and 3 years in the EJ subjects. However, in accordance with the "*guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products*" (EMEA/149995/2008 rev.1), the applicant has committed to follow up the subjects from the clinical programme up to 15 years in the planned post-authorisation study.

Co-Primary endpoint

ARSA Activity

For the overall treated population, the mean ARSA activity increased in the total PBMCs, BM and CSF to levels above (for PBMCs and BM) or within the range (for CSF) observed in healthy subjects within 3 months. The fact that in some patients ARSA activity was higher than in healthy subjects does not raise a safety concern because in the absence of substrate the enzyme is presumed to be inactive and non-clinical data indicate that supra-physiological ARSA activity levels in the PBMC and BM do not pose a risk.

At year 2 post treatment, the mean ARSA activity in the CSF was 0.85 nmol/mg/hr (95%CI 0.58; 1.25) for the *LI MLD* subjects and 0.64 nmol/mg/hr (95%CI 0.37; 1.13) for the *EJ MLD* subjects.

In the individual profiles fluctuations and decrease of ARSA activity levels in the CSF can be noted. The fluctuations seen are unlikely due to assay variability as this was reported to be low with inter-run and intra-run variability with a CV lower than 6%. It was hypothesised that fluctuations in protein content in CSF could theoretically drive fluctuations in reported ARSA activity in CSF and that the decrease observed in normalised ARSA activity in Late Infantile subjects at timepoints M60 and M96 might be driven by an increase in CSF protein. This could not be verified. For this a graph presenting CSF protein over time would have been helpful. As no new data on efficacy was submitted, it could also not be verified whether the decrease in ARSA activity led to a loss of efficacy. However, the applicant has committed to further investigate the relationship between CSF protein and ARSA activity to inform on maintenance of effect and loss of efficacy.

Gross Motor Function Measurement

For the *LI MLD* 2 years post-treatment, mean total GMFM score in the Libmeldy treated LI MLD subjects was 72.5 compared to 7.4 for the NHx subjects (Difference 65.1 points, 95%CI 41.6; 88.6), p<0.001).

The LI MLD group has one subject that was pre-symptomatic at screening and became symptomatic prior to treatment. This subject performed comparable to the NHx subjects, while the majority of the LI MLD showed performance within ranges observed for healthy subjects.

The trajectory of the matched control patients in the pre-symptomatic phase is mainly extrapolated based on the age of the patient, which does not take into account the phenotypic variability that characterises LI patients. However, the high proportion of siblings (7/9 treated LI patients had their sibling in the NHx group) provides reassurance on the validity of the matching exercise.

It is noted that for 3 of the 9 treated LI MLD subjects, deterioration in GMFM score is observed, which appears to be associated with a drop of ARSA activity level in the CSF. This observation raises a concern on the durability of the treatment effect in these subjects. The applicant indicates that fluctuation in ARSA are driven by an increase in CSF protein and no new data was submitted. However, as no new efficacy data was submitted it could not be verified whether the decrease in ARSA activity led to a loss of efficacy. Therefore, further follow up data will be gathered in as a post marketing commitment in the planned post-authorisation study.

For the overall *EJ* MLD subjects the mean total GMFM score at 2 years after treatment was 76.5 for the Libmeldy treatment group compared to 36.6 points for the historical cohort (Difference 39.8 points 95%CI 9.6; 80.1, p=0.026). However, due to the small number of subjects and high interpatient variability the comparison of mean values is not very informative.

The *pre-symptomatic EJ subjects* had an adjusted mean GMFM total score of 96.7%, while for the NHx a mean GMFM total score of 44.3% was reported. The difference between the Libmeldy treated and NHx subjects was 52.4% (95%CI 25.1; 79.6, P=0.008) at Year 2. The individual panel plots show that 3 of the 4 pre-symptomatic subjects performed in ranges of healthy subjects. One subject showed deterioration, which was accompanied by a decrease of ARSA activity in the CSF.

For the *symptomatic EJ subjects* the adjusted LS mean GMFM total score was 60.7% at year 2 post treatment. The difference from the NHx group was 28.7% (95% CI -14.1; 71.5, p=0.35) at year 2. At Year 3 there was a difference in treatment effect of 43.9% (59.8% vs. 15.9%; p=0.054). At Year 4 and Year 5 this

difference was confirmed (42.9% for both: 53.6% vs. 10.7% [p=0.054] and 50.3% vs. 7.4% [p=0-107], respectively). Although, on group level an effect is observed independent of symptomatic status, the individual profiles show predominantly deterioration on GMFM in the 8 symptomatic subjects.

Summary data and clarifications provided by the applicant indicated that the number of missing values is relatively small, although, given the already small sample size related to the rarity of the condition, missing values further reduce the actual numbers which the main efficacy analyses were based on. Concerning the NHx controls, it is acknowledged that, given the characteristics of this cohort, it was expected that not all subjects would have contributed to all analyses.

The co-primary efficacy endpoint, i.e. GMFM total score and ARSA activity, was assessed at a fixed time point of at 2 years post treatment. This is an observation at a single time point. With respect to the GMFM, the longitudinal evaluation is considered more appropriate, as this gives a better representation of the overall course of the efficacy in relation to the disease progression. For the majority of the LI-MLD the effect observed on the GMFM at 2 years is also reflected in the overall longitudinal pattern. In contrast, for all symptomatic EJ-MLD subjects a difference of 28.7% from the NHx is observed 2 years post treatment, while the individual panel plots showed deterioration. This deterioration was also observed in other outcomes, e.g. cognitive function, survival etc.

In addition, the relevance of interpretation of these endpoints on group level is limited as effects are diluted due to wide interpatient variability in these endpoints as it is evident from the individual panel plots for the different clinical outcomes. Moreover, the individual profiles for the symptomatic subjects indicate disease progression. It is unknown if this group shows a lack of efficacy due to reaching the point of no return. Therefore, other outcomes should be taken into account too.

In conclusion, looking at the individual trajectories of the disease course, for the majority of pre-symptomatic LI- and EJ MLD patients, a clinically relevant treatment effect is observed at the time point of the primary endpoint (2 years) which is maintained at later time points. However, significant deterioration is observed on motor function for symptomatic EJ MLD patients. While data are suggestive that this deterioration may be less rapid than in untreated subjects, this is still uncertain as the wide variability in the disease trajectory prohibits a final conclusion on this issue.

Note: treatment success was not discussed by the applicant, e.g. 2xSD ARSA activity and delay in progression of 10% GMFM. This issue is not pursued as the treatment effect is impressive, with pre-symptomatic subjects performing consistently within ranges reported for healthy subjects not only for motor assessment, but also for cognitive function.

Secondary outcomes

Brain MRI

For the *LI MLD* subject the MRI outcome was consistent with the GMFM scores, i.e. the MRI score mean differences between Libmeldy treated LI subjects and NHx subject of -11.8 ($p<0.001$). Also, individual plots show stabilisation and no deterioration over time.

For the *EJ MLD* on group level the difference between the Libmeldy treatment group and NHx was -4.1 point p=0.12 on brain MRI. For the *pre-symptomatic EJ* subjects, the differences in the adjusted LS mean MRI total scores between the Libmeldy treated pre-symptomatic EJ subjects and NHx subjects was 10.7 (95%CI 7.0, 14.4; $p<0.001$). For the *symptomatic EJ MLD* patients, the difference in LS mean MRI total score between the Libmeldy treated symptomatic subjects and NHX was 5.8 (95% CI -4.0, 15.5; P=0.21). Individual plots in symptomatic and pre-symptomatic EJ subjects show stabilisation over time.

The effects on morphology observed with the MRI do not correspond with the effects observed in the GMFM and other secondary outcomes. Therefore, the MRI on its own appears not to be an effective measure to determine efficacy of Libmeldy.

Cognitive performance

For the majority of the *LI MLD* subjects, the cognitive performance was within range reported for healthy subjects.

For the *EJ MLD* subjects, all 4 subjects who were treated prior to the onset of symptoms had neuropsychological composite scores that were largely within or above the normal range (score of 100 +/- SD of 15) at the time of the data cut-off, with the exception of the Processing Speed Index.

Throughout the extended follow-up period, 6/8 symptomatic EJ subjects who were early symptomatic, remained stable and above the threshold for severe cognitive impairment (IQ≥55) with a statistically significant difference from the NHx controls at Year 2 (IQ 88.9 vs. 31.9 in NHx controls; treatment difference 61.1, p=0.029), Year 3 (89.4 vs. 17.6; treatment difference 71.8, p=0.013), Year 4 (81.9 vs. 15.2; treatment difference 66.8, p=0.026), but not at Year 5 (48.2 vs. 9.8). In 4/5 subjects that would have met the current proposed treatment criteria (i.e. IQ>85 and GMFC-MLD ≤ 1) had cognitive function in the normal range of healthy subjects.

Survival

A borderline statistically significant difference was observed for overall survival of the *LI MLD* subjects as compared to the NHx cohort (unstratified Log-rank p-value 0.062) and for the *EJ subjects* (estimated HR 1.85; log rank p-value= 0.537). In the EJ subgroup 2 deaths occurred, which were related to the disease progression. Both concern patients that were early symptomatic at start of treatment.

The effect on survival is substantial, particularly in the pre-symptomatic patients. However, the quality of survival was not assessed by the applicant. This issue is not pursued as the pre-symptomatic subjects, show that the performance for both motor function and cognitive function is within the ranges of healthy subjects.

Correlation analyses between clinical outcomes and engraftment and PD parameters

As requested, the applicant performed a correlation analysis between engraftment (%LV+ cells in BM, VCN in PBMCs) and PD (ARSA activity in PBMCs and CSF) parameters and clinical efficacy outcome measures at Year 2 and Year 3 post-treatment. Statistically significant correlations were found between VCN and enzymatic activity in PBMCs at year 2 and Year 3 post-treatment. No correlation was found between engraftment parameters and ARSA activity in CSF; according to the applicant this could be due to the fact that ARSA activity in CSF could be an indirect marker of the enzyme secreted in the CNS and may underestimate the level of central enzymatic activity while the peripheral ARSA activity measured in PBMCs represents a more direct marker of the PD effects of Libmeldy treatment. Of note, conversely to what it could be expected, no statistically significant correlations were found between engraftment parameters and clinical outcome measures (i.e. GMFM at Year 2 and Year 3 timepoints post-treatment). This confirms that further studies are actually needed to better understand the etiopathogenesis and pathophysiology of MLD, the multiple factors involved in the Libmeldy treatment response, and the crucial role of the baseline patient clinical status.

A discrepancy between the lower VCN in patients compared with VCN in DP was observed. The applicant specified that the starting material of Libmeldy is comprised of heterogeneous CD34+ cell populations, mainly composed of committed, short-lived progenitor cells. A small fraction of those cells are long-lived stem cells which have the ability to self-renew, engraft and give rise to long-term repopulating lineages. There may be

a random and uneven distribution of successful transduction with LV between short- and long-lived CD34+ cells during manufacturing of Libmeldy. The applicant hypothesized that the VCN in the long-lived stem cell population may be lower than in the bulk CD34+ population and this could account for the difference between DP VCN and VCN in patients. The requested analyses performed by the applicant suggest that colonies with high VCN in DP engraft and high VCN in DP does not influence cell viability, clonogenic potential, and time to haematological reconstitution. Furthermore, as requested, the applicant evaluated potential effect of high ARSA activity in DP on cell viability by conducting a correlation analysis between ARSA activity in DP and clonogenic potential as a marker of clinical engraftment. A significant inverse correlation was found (corr=-0.45, p=0.004), however, according to the applicant, this was likely driven by two DP batches with a lower VCN and lower ARSA activity which had a higher clonogenic potential compared to the other 26 batches.

Overall efficacy

Overall, the difference in GMFM scores observed in the pivotal is substantial and is considered extremely clinically relevant. This observation in GMFM scores is further supported by other secondary outcomes. However, a large variability in treatment effect is noted across subpopulations with maintenance of GMFM scores/stabilisation in disease course seen in some patients and rapid deterioration in others.

The *LI MLD* subjects generally performed within ranges of healthy subjects for the different efficacy outcomes. For 3 of the 9 treated LI subjects, deterioration is observed, which coincide with a drop of ARSA activity levels. One LI-MLD subject became symptomatic just prior to treatment. This subject consistently shows deterioration in the different assessments.

For the *EJ MLD* population no clear relation between the ARSA activity levels in the CSF and clinical outcomes (e.g. GMFM, brain MRI, IQ) could be established.

Three of the *pre-symptomatic EJ MLD subjects* consistently performed within ranges reported for healthy subjects. One subject had levels below the minimum ARSA activity levels reported in the CSF for healthy subjects. These subjects consistently show deterioration.

All symptomatic patients irrespective of MLD variant (LI MLD (n=1), EJ MLD (n=3) showed deterioration across the motor function, cognitive function and parent reported outcomes. The degree of deterioration varied, which is consistent with the heterogeneity of MLD. In the early-symptomatic EJ-MLD subjects the cognitive function was maintained as subjects performed within or above ranges reported for healthy subjects. In light of the small study population studied and limited follow-up duration, a post-authorisation efficacy and safety study is required to evaluate the long-term efficacy of Libmeldy. It is considered necessary to obtain long-term follow up efficacy data to evaluate whether efficacy is maintained over time. Therefore, the study should also assess motor-functional development to track whether patients develop normally over time following Libmeldy treatment.

Because of these differences in efficacy seen in the subpopulations in the study, the analysis of efficacy across subgroups is important for support of the proposed indication.

Efficacy across subgroups.

Subgroup analyses were provided for disease subtype/variant (LI MLD/ EJ MLD), busulfan conditioning regimen (SMAC/MAC) and stage of the disease (Pre-symptomatic / symptomatic).

Disease subvariant (LI MLD vs EJ MLD)

Data from both the pivotal study (20 subjects, 9 LI MLD and 11 EJ MLD) and the full integrated efficacy set (33 subjects, 18 LI MLD and 15 EJ MLD) was presented for the LI MLD and EJ MLD population separately. The use of the full integrated set is agreed as treatment protocol was sufficiently similar and pooling of the data increases patient numbers. Further, as also indicated above, differences in disease progression that may impact the outcome as such analysis by subtype is endorsed.

Both groups showed a similar profile of ARSA activity in the PBMC, BM MNC and CSF, e.g. an increase within 28 Days to levels higher than reported for healthy subjects in the PBMC and BM MNC and with levels within the range reported for healthy subjects. However, the applicant acknowledged that from 6 months to Year 6 post-treatment, efficacy results in terms of geometric mean of LV+ values cells in BM, VCN in BM-derived CD34+ cells and in PBMCs, ARSA activity in PBMCs were better with higher and, in some cases, relatively more stable values in the LI subgroup compared with the EJ subgroup. In any case, there is an overlap between the ranges reported for the LI subjects and the EJ subjects. Considering the mode of action and the same underlying pathophysiology, this is not unexpected. It is agreed with the applicant that, as the sample size at later timepoints was small, the results on the assessment of the long-term efficacy should be interpreted with caution.

The effect on GMFM, i.e. differences in score from the natural history cohort, was greater for the LI MLD population than for the EJ MLD population. However, differences on a group level appear to be driven by a difference in the symptomatic status of the subjects between the two populations.

Concerning the long-term persistence of treatment effect, the reduction in the engraftment parameters (i.e. mean proportion of LV+ cells in BM over time) observed in both MLD variant subgroups did not have an impact on pharmacodynamic effect of Libmeldy treatment in terms of ARSA activity and apparently on clinical efficacy outcome measures. The applicant did not consider a second transplant as applicable and necessary because of i) the limited correlations between levels of engraftment and clinical outcomes, ii) the role of symptomatic status at the time of treatment that has a great impact on patient outcome after treatment, iii) the risk of insertional oncogenesis that after a second transplant is unknown and could increase, and finally iv) the benefit-risk profile of a second conditioning regimen such as that applied for Libmeldy treatment that is unknown. These argumentations for the need to administer Libmeldy once are reasonable and acceptable.

Disease stage (pre-symptomatic vs Symptomatic)

In the PK/PD section above data by symptomatic status was also presented. In brief, there was no effect of symptomatic status on the engraftment of Lentiviral vector positive cells or ARSA levels achieved in the PBMC, BM MNC and CSF. However, the clinical outcomes show an effect of symptomatic status.

In the LI MLD group all subjects were included pre-symptomatically, however, one subject became symptomatic prior to treatment. This subject consistently performed within ranges and close to the natural history cohort for all outcomes, while the pre-symptomatic LI MLD subjects performed within or close to the ranges of healthy subjects Indicating that for this symptomatic LI-MLD subject the window of opportunity to perceive treatment benefit on either motor function or cognitive function was missed. Therefore, treatment in LI-MLD should be initiated before subjects become symptomatic.

Pre-symptomatic EJ MLD subjects *(n=4)* had an adjusted mean GMFM total score of 96.7%, while for the NHx a mean GMFM total score of 44.3% was reported. The difference between the Libmeldy treated and NHx subjects was 52.4% (95%CI 25.1; 79.6, P=0.008) at Year 2. The differences in the adjusted LS mean MRI total scores between the Libmeldy treated pre-symptomatic EJ subjects and NHx subjects was 10.7 (95%CI 7.0,14.4; p<0.001). At the time of the data cut-off, with the exception of the Processing Speed Index, all 4

subjects who were treated prior to the onset of symptoms had neuropsychological composite scores that were largely within or above the normal range (score of 100 +/- SD of 15).

For the *symptomatic EJ* subjects the adjusted LS mean GMFM total score was 60.7% at year 2 post treatment. Difference from the NHx group was 28.7% (95% CI -14.1; 71.5, p=0.35) at year 2. The difference in LS mean MRI total score between the Libmeldy treated symptomatic subjects and NHX was 5.8 (95% CI -4.0, 15.5; P=0.21). Four of the 7 subjects had total IQ above the severe mental disability threshold (IQ>55) at Year 2 and Year 3 post-GT. In 3 of the 4 subjects the test could not be performed. Data on cognitive function shows that treated early symptomatic EJ MLD subjects perform within ranges of healthy children. This indicates a window of opportunity for treatment as cognitive function remains intact over the period analysed.

Data showed that, while pre-symptomatic patients have a better response to Libmeldy treatment compared with the symptomatic subjects (mainly early symptomatic EJ subjects in the case of the clinical development programme of Libmeldy), these last experienced a disease progression following treatment. For treated early symptomatic EJ subjects it appears that there is a delay in rate of motor function deterioration and that cognitive function is maintained, indicating a window of opportunity for symptomatic EJ MLD population. The applicant proposes that treatment should be initiated before in symptomatic MLD patients aged 30 months to 7 years with an IQ score ≥85 or GMFC-MLD<1. This cut off point is accepted. If more follow up data becomes available these criteria may need to be adapted. Further identification of prognostic factors for response, i.e. target population, will be part of the post-authorisation study

In conclusion, efficacy in pre-symptomatic LI-MLD and EJ-MLD and early symptomatic EJ-MLD is demonstrated.

Conditioning regimen

Two different busulfan conditioning regimens were used (SMAC and MAC) and the effect of the conditioning regimen on transduced cell engraftment and ARSA activity are discussed in the Pharmacodynamics section.

Supportive studies.

The effect observed in the expanded programme studies reflects the outcomes seen in the pivotal study. In the expanded excess programmes only pre-symptomatic patients were treated.

One study focused on the effect of the fresh formulation (Libmeldy-f) compared to the commercial formulation (Libmeldy-c, cryopreserved). Although the clinical data is limited (n=4), no differences is observed in the short-term effect (e.g. 3 months-1year) on GMFM, ARSA CSF activity and other functional outcome measures. The outcomes were consistent with the pivotal study.

Proposed post-authorisation plan

Limited data was provided by the applicant in terms of number of subjects and follow up time. Therefore, the applicant has committed to conduct a post authorisation efficacy and safety study LongTERM-MLD. The main goal of proposed study LongTERM-MLD is to increase the patient numbers and to provide long term follow up data (up to 15 years). The applicant proposes to enrol two groups of patients:

Group 1: all patients who are treated in a clinical programme of Libmeldy, i.e. patients who already received treatment will be followed up to 15 years and patients enrolled in a new clinical study. This group will

therefore also include subjects with LJ-MLD as a study in this population is open for recruitment. Group 2: all patients treated in a post marketing setting, i.e. LI MLD and EJ MLD subjects. The applicant will include Off-label treated subjects as a separate subgroup 2c.

The CAT agreed that this study is needed to ensure long-term follow-up of efficacy and safety of Libmeldy treatment. In addition, the applicant should submit a full study protocol within 3 months after approval, thus a detailed assessment of the proposed study will be performed at a later stage.

Extrapolation to full paediatric MLD variant.

Based on the comments received regarding the full extrapolation from LI-MLD and EJ-MLD to LJ-MLD, the applicant has removed the LJ-MLD from the indication. Full extrapolation is not agreed as the disease progression of LJ-MLD is different to that from LI-MLD and EJ-MLD, and much closer to the adult-MLD. Therefore, the benefit/risk should be weighed differently. Moreover, LJ-MLD patients are currently identified when already symptomatic. As the symptomatic status impacts the efficacy, the prognostic parameters for treatment success in the LJ-MLD populations should be identified and substantiated. Therefore, the applicant intends to submit data from an open label non-randomized study to extend the indication to LJ-MLD subjects.

Additional expert consultation

Not Applicable

2.7.2. Conclusions on clinical efficacy

Libmeldy shows impressive efficacy in the pre-symptomatic LI MLD and EJ MLD as physical and cognitive performance is within the normal range of healthy subjects for the vast majority of the pre-symptomatically treated subjects for the duration of FU. The median follow-up at time of submission (cut-off date March 2018) for the pivotal study was 5.4 years (range: 2.98 to 7.51 years) and 3.5 years (range: 0.64 to 6.55 years) for LI MLD (n=9) and EJ MLD (n=11), respectively. The effects of the treatment are evident on gross motor function, cognitive function, brain MRI, and survival. As there appears a reduction in cells with high VCN and the ARSA levels seems to decrease over time, it remains to be seen whether the effect of treatment is maintained or whether, in time, progression to symptomatic disease may occur. However, further data can be gathered post marketing to confirm efficacy as the current numbers are limited, and to reassure a maintenance of effect as the follow up will be 15 years.

While deterioration on motor function is observed in all symptomatic EJ subjects, data indicate that for the early symptomatic patients there appears to be a delay in rate of motor function deterioration and the cognitive function is within or above the ranges of heathy subjects form the same age. Also, as there is currently no screening programme for MLD, the majority of subjects will be identified when they are symptomatic. The applicant aimed to identify the “cut-off” points beyond which the window of opportunity for treatment is lost for symptomatic EJ MLD patients. The proposed cut off point is accepted. If more follow up data becomes available, these criteria may need to be adapted. Further identification of prognostic factors for response, i.e. target population, should be part of the Post authorisation safety and efficacy study.

The CAT considered the following measures necessary to ensure the follow-up of efficacy:

In order to further characterise the long-term efficacy and safety of Libmeldy in children with late infantile or early juvenile forms of MLD, the MAH will conduct and submit the results of a prospective study based on data from a registry, according to an agreed protocol.

The CHMP endorse the CAT conclusion on clinical efficacy as described above.

2.8. Clinical safety

Patient exposure

The safety of Libmeldy was evaluated in 2 studies and in the expanded access programmes.

The safety populations have been defined by the applicant as follows:

- Safety set (n= 29) for the integrated analysis. Includes patients from:
  - Registrational Study 201222 (n= 20)
  - Expanded Access Programs (n= 9):
    - a single patient from the Compassionate Use Programme (CUP) (207394)
    - Hospital Exemption (HE) programme (205029 n=3 patients)
    - CUP (206258 n=5 patients)
- All subjects set (n= 30) includes all enrolled subjects across all clinical studies.

Data are presented separately for 4 subjects treated with Libmeldy-c in the ongoing Study 205756.

The data cut-offs for safety are as follows: 30 March 2018 (registrational study 201222), 5 January 2018 / 5 December 2018 / 5 December 2018 (expanded access programme) and 14 March 2019 (study 205756).

Study phases

AEs were prospectively reported in the Libmeldy clinical development programme. However, after data collection, AEs were retrospectively allocated to the following study phases to facilitate the evaluation of the short- and long-term safety of treatment with Libmeldy; AEs were summarized and presented according to the following time periods by the applicant, unless otherwise specified:

- *Pre-treatment phase*: Defined as prior to the first day of the conditioning regimen. Includes Screening and Baseline.
- *Treatment phase*: From the first day of the conditioning regimen to the date of GT infusion. As a conservative approach, events that occurred on the same day as the first day of the conditioning regimen were included in the treatment phase as were any events that occurred on the date of GT infusion itself (Day 1).
- *Follow-up phase*: Comprises the whole post-GT period, and was divided into the following sub-phases for evaluation of safety:

- *Acute phase*: the period up to 48 hours after the end of the GT infusion (i.e. Day 2 and Day 3, inclusive).
- *3-month post-GT*: from 48 hours after the end of GT infusion up to and including Day 100.
- *Short-term phase*: ≥ Day 101 and < Day 1098.
- *Long-term phase*: ≥ Day 1098.

In any given phase, only those AEs that started during that phase are reported. The AEs that were reported in previous phase(s) and were ongoing at start of the subsequent phase(s) were not reported in subsequent phase(s).

Duration of exposure

At the time of initial submission, the Integrated Safety Set consisted of 29 subjects with a median duration of follow-up of 3.160 years (range 0.64 to 7.51 years) (Table 11). The median duration of follow-up was similar in the LI subgroup (3.035 years) and in the EJ subgroup (3.49 years). Two LI subjects had completed more than 7 years of follow-up.

Table 11: Duration of Follow-up (All Subjects)

	Late Infantile (N=16)	Early Juvenile (N=14[a])	Total (N=30[a])
Duration of Follow-Up (years)			
n	16	13	29
Mean (SD)	3.829 (2.2637)	3.402 (1.6844)	3.638 (2.0019)
Median (Min, Max)	3.035 (0.99, 7.51)	3.490 (0.64, 6.55)	3.160 (0.64, 7.51)
Duration of Follow-Up Category			
n	16	13	29
≥6 months	16 (100)	13 (100)	29 (100)
≥1 year	15 (94)	12 (92)	27 (93)
≥2 years	12 (75)	10 (77)	22 (76)
≥3 years	8 (50)	8 (62)	16 (55)
≥4 years	7 (44)	4 (31)	11 (38)
≥5 years	6 (38)	2 (15)	8 (28)
≥6 years	4 (25)	1 (8)	5 (17)
≥7 years	2 (13)	0	2 (7)
≥8 years	0	0	0

[a] One subject (in Study 201222) was withdrawn by the investigator at the Baseline visit (prior to receiving Libmeldy-f) due to rapid disease progression.

Abbreviations: Max=maximum; Min-minimum; SD=standard deviation

Exposure busulfan conditioning

In the Integrated Safety Set, 13 subjects (45%) were treated with a SMAC regimen, defined as a target cumulative AUC of 67,200 µg*h/L (target range 58,800 to 78,400 µg*h/L). Sixteen subjects (55%) were administered the MAC regimen, defined as a target cumulative AUC of 85,000 µg*h/L (target range: 76,500 to 93,500 µg*h/L). Please refer to section 3.3.1 for the exposure results of the busulfan conditioning.

Adverse events

Integrated safety set

Table 12 depicts the most frequently reported adverse events across the treatment phases. The most frequently reported adverse events in the follow-up phase (post gene therapy) were infections and infestations (90% of subjects), blood and lymphatic system disorders (79% of subjects), gastrointestinal disorders (79% of subjects), investigations (79% of subjects), general disorders and administration site conditions (76% of subjects), hepatobiliary disorders (55% of subjects), and nervous system disorders (52% of subjects).

Table 12: Adverse Events Reported in 3 or More Subjects (at least 10%) in the Follow-up Post-GT Phase, by Preferred Term and Treatment Phase (Integrated Safety Set)

Phase	Pre-Tx	Tx	Acute	3 Month Post-GT	Short Term	Long Term	Total Follow-up Post-GT
PT	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=16) n (%)	(N=29) n (%)
Any Event	29 (100)	17 (59)	3 (10)	28 (97)	28 (97)	13 (81)	29 (100)
Febrile Neutropenia	0	0	0	23 (79)	0	0	23 (79)
Gait Disturbance	1 (3)	0	0	5 (17)	9 (31)	1 (6)	15 (52)
Upper Respiratory Tract Infection	7 (24)	0	0	3 (10)	11 (38)	5 (31)	14 (48)
Blood IgE Increased	4 (14)	0	0	6 (21)	6 (21)	1 (6)	13 (45)
Stomatitis	0	0	0	12 (41)	0	0	12 (41)
Mucosal Inflammation	0	0	0	10 (34)	0	0	10 (34)

Phase	Pre-Tx	Tx	Acute	3 Month Post-GT	Short Term	Long Term	Total Follow-up Post-GT
Device Related Infection	3 (10)	0	0	4 (14)	5 (17)	0	9 (31)
Motor Dysfunction	0	0	0	2 (7)	7 (24)	0	9 (31)
Muscle Spasticity	0	0	0	1 (3)	6 (21)	2 (13)	9 (31)
Ear Infection	0	0	0	1 (3)	7 (24)	0	7 (24)
Pyrexia	0	0	0	1 (3)	5 (17)	3 (19)	7 (24)
Vitamin D Decreased	0	0	0	0	3 (10%)	4 (25)	7 (24)
Aphasia	0	0	0	1 (3)	4 (14)	1 (6)	6 (21)
Conjunctivitis	0	0	0	1 (3)	5 (17)	1 (6)	6 (21)
Enteritis	0	0	0	0	6 (21)	0	6 (21)
Rash Erythematous	0	1 (3)	0	6 (21)	0	0	6 (21)
Serum Ferritin Increased	0	0	0	6 (21)	0	0	6 (21)
Vomiting	1 (3)	0	0	3 (10)	2 (7)	1 (6)	6 (21)
Ataxia	0	0	0	2 (7)	3 (10)	0	5 (17)
Dysarthria	0	0	0	1 (3)	4 (14)	0	5 (17)
Neutropenia[a]	0	0	0	5 (17)	0	0	5 (17)
Antibody Test Positive	0	0	0	2 (7)	3 (10)	0	4 (14)
Body Mass Index Decreased	2 (7)	0	0	0	3 (10)	1 (6)	4 (14)
Cognitive Disorder	0	0	0	0	3 (10)	1 (6)	4 (14)
Dysphagia	0	0	0	0	3 (10)	1 (6)	4 (14)
Gall Bladder Polyp	2 (7)	0	0	0	4 (14)	0	4 (14)
Gastroenteritis	0	0	0	0	3 (10)	1 (6)	4 (14)

Phase	Pre-Tx	Tx	Acute	3 Month Post-GT	Short Term	Long Term	Total Follow-up Post-GT
Head Injury	0	1 (3)	0	2 (7)	0	2 (13)	4 (14)
Hepatomegaly	0	2 (7)	1 (3)	3 (10)	0	0	4 (14)
Metabolic Acidosis	3 (10)	4 (14)	0	2 (7)	1 (3)	1 (6)	4 (14)
Pneumonia	0	0	0	1 (3)	3 (10)	1 (6)	4 (14)
Clostridium Difficile Colitis	0	0	0	3 (10)	0	0	3 (10)
Epistaxis	0	0	0	2 (7)	1 (3)	0	3 (10)
Gall Bladder Enlargement	22 (76)	0	0	1 (3)	1 (3)	1 (6)	3 (10)
Influenza Like Illness	0	0	0	0	3 (10)	0	3 (10)
Osteoporosis	0	0	0	0	1 (3)	2 (13)	3 (10)
Phimosis	0	0	0	0	2 (7)	1 (6)	3 (10)
Respiratory Tract Infection	0	1 (3)	0	0	2 (7)	1 (6)	3 (10)
Scarlet Fever	0	0	0	0	1 (3)	2 (13)	3 (10)
Urinary Tract Infection	0	0	0	1 (3)	2 (7)	0	3 (10)
Varicella	0	0	0	1 (3)	1 (3)	1 (6)	3 (10)
Venooclusive Liver Disease	0	0	0	3 (10)	0	0	3 (10)
Viral Infection	0	0	0	1 (3)	1 (3)	1 (6)	3 (10)

[a] Per protocol, neutropenia occurring within the first 3 months post-GT was only reported as an AE if National Cancer Institute Common Toxicity Criteria was at least Grade 3.
Abbreviations: GT=gene therapy; IgE=immunoglobulin E; PT=preferred term; Tx=treatment.

Study 205756

Most of the AEs observed during the Follow-up phase occurred within the 3-month post-treatment phase (34 of 41 events). All subjects had at least 1 AE during the 3-month post-treatment phase. The most common AEs during this phase were febrile neutropenia, neutropenia, and stomatitis (see Table 13 below).

Table 13: Summary of Adverse Events by Treatment Phase (Number of Subjects and Occurrences)

System Organ Class Preferred Term	Pre-Treatment (N=4)	Treatment (N=4)	Acute (N=4)	3 Months Post-Treatment (N=4)	Short-Term (N=2)	Long-Term (N=0)	Follow-Up (N=4)
	Number of Subjects with Adverse Events (%) [Number of Events]						
Any Event	4 (100) [21]	1 (25) [3]	0	4 (100) [34]	2 (100) [7]	0	4 (100) [41]
Blood and Lymphatic System Disorders							
Febrile Neutropenia	0	0	0	4 (100) [5]	0	0	4 (100) [5]
Neutropenia[a]	0	0	0	3 (75) [4]	0	0	3 (75) [4]
Gastrointestinal Disorders							
Stomatitis	0	0	0	3 (75) [3]	0	0	3 (75) [3]
Diarrhoea	1 (25) [1]	0	0	1 (25) [1]	0	0	1 (25) [1]
Constipation	1 (25) [1]	0	0	0	0	0	0
General Disorders and Administration Site Conditions							
Pyrexia	1 (25) [1]	0	0	2 (50) [2]	1 (50) [1]	0	2 (50) [3]
Gait Disturbance	0	0	0	0	1 (50) [1]	0	1 (25) [1]
Hepatobiliary Disorders							
Cholecystitis Acute	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Gallbladder Enlargement	2 (50) [2]	0	0	1 (25) [1]	0	0	1 (25) [1]
Hypertransaminasaemia	0	1 (25) [1]	0	0	0	0	0
Infections and Infestations							
Enterovirus Infection	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Gastroenteritis	0	0	0	0	1 (50) [1]	0	1 (25) [1]
Herpes Zoster	0	0	0	0	1 (50) [1]	0	1 (25) [1]
Klebsiella Infection	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Nasopharyngitis	0	0	0	0	1 (50) [1]	0	1 (25) [1]
Otitis Media	0	0	0	0	1 (50) [1]	0	1 (25) [1]
Pharyngitis	0	0	0	0	1 (50) [1]	0	1 (25) [1]
Sepsis	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Adenovirus Infection	1 (25) [1]	0	0	0	0	0	0
Cytomegalovirus Infection	0	1 (25) [1]	0	0	0	0	0
Device Related Infection	2 (50) [2]	0	0	0	0	0	0
Haemophilus Infection	1 (25) [1]	0	0	0	0	0	0
Helicobacter Infection	1 (25) [1]	0	0	0	0	0	0

System Organ Class Preferred Term	Pre-Treatment (N=4)	Treatment (N=4)	Acute (N=4)	3 Months Post-Treatment (N=4)	Short-Term (N=2)	Long-Term (N=0)	Follow-Up (N=4)
	Number of Subjects with Adverse Events (%) [Number of Events]						
Upper Respiratory Tract Infection	1 (25) [1]	0	0	0	0	0	0
Injury, Poisoning and Procedural Complications							
Arthropod Bite	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Transfusion Reaction	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Investigations							
Antithrombin III Decreased	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Cytomegalovirus Test Positive	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Roseolovirus Test Positive	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Blood Immunoglobulin E Increased	2 (50) [2]	0	0	0	0	0	0
Giardia Test Positive	0	1 (25) [1]	0	0	0	0	0
Herpes Simplex Test Positive	1 (25) [1]	0	0	0	0	0	0
Oxygen Saturation Decreased	1 (25) [1]	0	0	0	0	0	0
Staphylococcus Test Positive	1 (25) [1]	0	0	0	0	0	0
Metabolism and Nutrition Disorders							
Metabolic Acidosis	1 (25) [1]	0	0	0	0	0	0
Musculoskeletal and Connective Tissue Disorders							
Arthralgia	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Renal and Urinary Disorders							
Renal Tubular Acidosis	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Respiratory, Thoracic and Mediastinal Disorders							
Respiratory Distress	1 (25) [1]	0	0	1 (25) [1]	0	0	1 (25) [1]
Rhinorrhoea	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Skin and Subcutaneous Tissue Disorders							
Rash Erythematous	0	0	0	1 (25) [2]	0	0	1 (25) [2]
Hyperkeratosis	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Rash	0	0	0	1 (25) [1]	0	0	1 (25) [1]
Rash Maculo-Papular	1 (25) [1]	0	0	1 (25) [1]	0	0	1 (25) [1]
Drug Eruption	1 (25) [1]	0	0	0	0	0	0

[a] Neutropenia AE refers only to Prolonged Neutropenia – ANC <500 µL at Day +45.
Abbreviations: AE=adverse event; ANC=absolute neutrophil count

Adverse reactions related to Libmeldy

Four subjects (14%) experienced adverse reactions related to Libmeldy. These events were reported as the preferred term 'ARSA antibody test positive' (4 subjects, 14%).

Adverse reactions potentially attributable to myeloablative conditioning (busulfan)

The review process for the selection of adverse drug reactions (ADRs) potentially attributable to myeloablative conditioning was conducted using the integrated data set and was developed to ensure consideration of many aspects of the data.

The specific process included several steps. First, the preferred terms were further reviewed and compared with the product characteristics of busulfan (Busilvex, SmPC; Busulfex, US Prescribing Information) and other gene therapies (Strimvelis, SmPC; Zynteglo, SmPC) where similar myeloablative conditioning regimens have been used. AEs were flagged at the preferred term level, and then an iterative process involving the principles above was applied to the preferred terms in each SOC. Collectively, 39 preferred terms were flagged as potentially attributable to myeloablative conditioning.

Second, for the flagged preferred terms, further review of subject-level data was performed, including an assessment of relevant medical history, co-morbidities, and other AEs.

Finally, there was a clinical evaluation, which included, as appropriate, consideration of similar preferred terms, biological plausibility, nature and timing of the events, the underlying disease, and incidence of the event in the pediatric population. After a comprehensive final assessment, 33 preferred terms from 14 SOCs were determined by the sponsor to be potentially attributable to myeloablative conditioning (Table 14).

Table 14: Adverse Events Potentially Attributable to Myeloablative Conditioning (Busulfan)

System Organ Class	Very Common >10%	Common <10%
Blood and Lymphatic System Disorders	Febrile Neutropenia, Neutropenia	Anaemia, Thrombocytopenia
Gastrointestinal Disorders	Stomatitis, Vomiting	Ascites, Diarrhoea, Gastrointestinal haemorrhage, Nausea
General Disorders and Administration Site Conditions	-	Pyrexia
Hepatobiliary Disorders	Hepatomegaly, Venoocclusive liver disease	Hypertransaminasaemia
Infections and Infestations		Cytomegalovirus viraemia, Pneumonia, Staphylococcal infection, Urinary tract infection, Viral infection
Investigations		Alanine aminotransferase increased, Aspartate aminotransferase increased, Aspergillus test positive

System Organ Class	Very Common >10%	Common <10%
Metabolism and Nutrition Disorders	Metabolic acidosis	Fluid overload
Musculoskeletal and Connective Tissue Disorders		Back pain, Bone pain
Nervous System Disorders		Headache
Psychiatric Disorders		Insomnia
Renal and Urinary Disorders		Oliguria
Reproductive System and Breast Disorders	Ovarian failure	
Respiratory, Thoracic and Mediastinal Disorders		Epistaxis, Oropharyngeal pain
Skin and subcutaneous tissue disorders		Skin exfoliation

Adverse reactions potentially attributable to MLD

Symptoms of MLD (not pre-defined) were reported only if clinically significant and NCI CTC Grade ≥3. AEs were manually reviewed by the sponsor and confirmed by the investigators after database lock to identify AEs typically associated with symptoms of MLD (e.g. ataxia, motor impairment, muscle spasticity, dysphagia). The decision to classify an event as associated with MLD was based on clinical judgement and experience with MLD.

During the Follow-up post-GT phase, in addition to the events of renal tubular acidosis and metabolic acidosis, events associated with MLD included gait disturbance (15 subjects, 52%), motor dysfunction (9 subjects, 31%), muscle spasticity (9 subjects, 31%), aphasia (6 subjects 21%), ataxia (5 subjects, 17%), dysarthria (5 subjects, 17%), cognitive disorder (4 subjects, 14%), dysphagia (4 subjects, 14%), and seizure (2 subjects, 7%).

In Study 205756, there was only one AE classified as associated with MLD, which was gait disturbance in one subject (Patient 30) at Month 6 (age 18.7 months) and Month 9 (age 22.1 months) due to a delay in independent walking.

Serious adverse events and deaths

Serious adverse events

Integrated safety set

Adverse events by maximum grade

All subjects (100%) in the Integrated Safety Set experienced at least one Grade 3 or higher AE (see Table 15). The most frequently reported (>50% of subjects) Grade 3 events were febrile neutropenia (79% of subjects), gait disturbance (52% of subjects), and stomatitis (41% of subjects). Four subjects (14%)

experienced Grade 4 events, including dysphagia in two subjects, metabolic acidosis in one subject, and VOD and atypical hemolytic uremic syndrome in one subject.

Table 15: Grade 3 or Higher Adverse Events Reported in 2 or More Subjects (at least 7%) in the Follow-Up Post-GT Phase, by Preferred Term by Study Phase (Integrated Safety Set)

PT	Pre-Tx	Tx	Acute	3 Month Post-GT	Short Term	Long Term	Total Follow-up Post-GT
	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=16) n (%)	(N=29) n (%)
Any Event	7 (24)	8 (28)	0	27 (93)	22 (76)	5 (31)	29 (100)
Febrile Neutropenia	0	0	0	23 (79)	0	0	23 (79)
Gait Disturbance	1 (3)	0	0	5 (17)	9 (31)	1 (6)	15 (52)
Stomatitis	0	0	0	12 (41)	0	0	12 (41)
Motor Dysfunction	0	0	0	2 (7)	7 (24)	0	9 (31)
Muscle Spasticity	0	0	0	1 (3)	6 (21)	2 (13)	9 (31)
Mucosal Inflammation	0	0	0	9 (31)	0	0	9 (31)
Aphasia	0	0	0	1 (3)	4 (14)	1 (6)	6 (21)
Ataxia	0	0	0	2 (7)	3 (10)	0	5 (17)
Device Related Infection	3 (10)	0	0	2 (7)	3 (10)	0	5 (17)
Neutropenia[a]	0	0	0	5 (17)	0	0	5 (17)
Cognitive Disorder	0	0	0	0	3 (10)	1 (6)	4 (14)
Dysarthria	0	0	0	1 (3)	4 (14)	0	5 (17)
Dysphagia	0	0	0	0	3 (10)	1 (6)	4 (14)
Vomiting	0	0	0	3 (10)	0	1 (6)	4 (14)
Enteritis	0	0	0	0	3 (10)	0	3 (10)
Metabolic Acidosis	2 (7)	4 (14)	0	2 (7)	1 (3)	0	3 (10)
Pneumonia	0	0	0	1 (3)	2 (7)	1 (6)	3 (10)
Venooclusive Liver Disease	0	0	0	3 (10)	0	0	3 (10)
Atypical Haemolytic Uraemic Syndrome	0	0	0	2 (7)	0	0	2 (7)
Clostridium Difficile Colitis	0	0	0	2 (7)	0	0	2 (7)
Epistaxis	0	0	0	2 (7)	0	0	2 (7)
Rash Erythematous	0	0	0	2 (7)	0	0	2 (7)
Seizure	0	0	0	0	1 (3)	1 (6)	2 (7)

[a] Prolonged neutropenia (neutropenia beyond Day 45 post-treatment)
Abbreviations: GT=gene therapy; PT=preferred term; Tx=treatment.

Serious adverse events

In the Integrated Safety Set, 20 subjects (69%) experienced SAEs during the Follow-up post-GT phase. SAEs were most frequently reported in the gastrointestinal disorders (31% of subjects), infections and infestations (28% of subjects), and nervous system disorders (21% of subjects) SOCs (See Table 16).

Table 16: Serious Adverse Events by Study Phase (Integrated Safety Set)

PT	Pre-Tx	Tx	Acute	3 Month Post-GT	Short Term	Long Term	Total Follow-up Post-GT
	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=29) n (%)	(N=16) n (%)	(N=29) n (%)
Any Event	2 (7)	0	0	5 (17)	16 (55)	3 (19)	20 (69)
Dysphagia	0	0	0	0	3 (10)	1 (6)	4 (14)
Motor Dysfunction	0	0	0	1 (3)	3 (10)	0	4 (14)
Vomiting	0	0	0	2 (7)	0	1 (6)	3 (10)
Device-related Infection	2 (7)	0	0	0	2 (7)	0	2 (7)
Enteritis	0	0	0	0	2 (7)	0	2 (7)
Gallbladder Polyp	0	0	0	0	2 (7)	0	2 (7)
Metabolic Acidosis	0	0	0	1 (3)	1 (3)	0	2 (7)
Muscle Spasticity	0	0	0	1 (3)	1 (3)	0	2 (7)
Pneumonia	0	0	0	0	2 (7)	0	2 (7)
Respiratory Tract Infection	0	0	0	0	1 (3)	1 (6)	2 (7)
Seizure	0	0	0	0	1 (3)	1 (6)	2 (7)
Anaemia	0	0	0	1 (3)	0	0	1 (3)
Atypical Haemolytic Uraemic Syndrome	0	0	0	1 (3	0	0	1 (3)
Bacterial Sepsis	0	0	0	0	1 (3)	0	1 (3)
Escherichia Infection	0	0	0	0	1 (3)	0	1 (3)
Foot Deformity	0	0	0	0	1 (3)	0	1 (3)
Gastroenteritis	0	0	0	0	1 (3)	0	1 (3)

PT	Pre-Tx	Tx	Acute	3 Month Post-GT	Short Term	Long Term	Total Follow-up Post-GT
Gastroenteritis Rotavirus	0	0	0	0	1 (3)	0	1 (3)
Ischaemic Cerebral Infarction	0	0	0	0	1 (3)	0	1 (3)
Kawasaki's Disease	0	0	0	0	1 (3)	0	1 (3)
Lung Infection	0	0	0	0	1 (3)	0	1 (3)
Pneumonia Aspiration	0	0	0	0	1 (3)	0	1 (3)
Status Epilepticus	0	0	0	0	1 (3)	0	1(3)
Thrombocytopenia	0	0	0	1 (3)	0	0	1 (3)
Venooclusive Liver Disease	0	0	0	1 (3)	0	0	1 (3)

Abbreviations: GT=gene therapy; PT=preferred term; Tx=treatment

Study 205756

By maximum grade

In Study 205756, 4 subjects (100%) experienced a total of 19 Grade 3 AEs. During the Treatment phase, 1 subject (Patient 32) experienced a Grade 3 AE of hypertransaminasemia. During the 3-month post-treatment phase, all 4 subjects (100%) experienced Grade 3 AEs. Febrile neutropenia (4 subjects, 5 events), neutropenia (3 subjects, 3 events), and stomatitis (3 subjects, 3 events) were the only AEs that occurred in >1 subject. One subject (Patient 30) experienced Grade 3 AEs of febrile neutropenia and neutropenia. One subject (Patient 31) had Grade 3 AEs of stomatitis, febrile neutropenia, and neutropenia, and an SAE of Grade 3 sepsis. One subject (Patient 32) had Grade 3 AEs of stomatitis, febrile neutropenia (x2), and neutropenia. One subject (Patient 33), who only had follow-up data until Month 1, had Grade 3 AEs of febrile neutropenia, stomatitis, and acute cholecystitis. Two subjects had follow-up data in the Short-term phase. Of those subjects, only one subject (Patient 30), who had follow-up data to Year 1, had a Grade 3 AE of gait disturbance. No Grade 4 or Grade 5 AEs were reported during any of the study phases in Study 205756.

Serious adverse events

In Study 205756, a total of 4 SAEs were reported in two subjects. One SAE was reported after treatment with Libmeldy-c, an event of sepsis that occurred within the 3-month post-treatment phase. Three of the SAEs occurred in the Pre-treatment phase (device-related infection n=2, respiratory distress n=1). None of the SAEs were considered by the investigator to be related to Libmeldy-c.

Deaths

To date, three deaths have been reported in subjects treated with Libmeldy during the clinical development programme (two subjects -Patient 19 and Patient20- in Study 201222; one subject -Patient 27- in Study 206258), all deemed to be unrelated to Libmeldy-f.

Two of these deaths were attributed to rapid progression of the underlying disease; in both cases the subjects were reported to be early symptomatic at the time of GT. One death was due to left hemisphere cerebral ischemic stroke, deemed unrelated to Libmeldy-f. All three events are further described below.

Two subjects (Patient 19,20) died during the registrational study as a result of events associated with rapid disease progression (dysphagia) approximately 15 and 8 months, respectively, after receiving treatment.

One subject (Patient 19) was diagnosed with EJ MLD at 5 years of age and was treated at an early symptomatic stage. Post-GT, motor and cognitive function continued to deteriorate. By 5 months post-GT, the subject's motor function was limited with the ability to stand with support and crawl a few meters, with an estimated Gross Motor Function Classification in MLD level 2 to 3. Difficulty in swallowing was first noted at 6 months post-GT, and motor dysfunction was reported as serious by 9 months post-GT. At approximately 14 months after treatment, the subject experienced worsening spasticity (NCI CTCAE Grade 4) and dysphagia (NCI CTCAE Grade 5). The parents declined placement of a PEG feeding tube. The subject died approximately 15 months after receiving treatment. The investigator considered the death to be due to disease progression.

The other subject (Patient 20) was diagnosed with EJ MLD at 5 years of age and treated at an early symptomatic stage. Post-GT, progressive difficulties in walking and slightly slower speech were observed. At 5 months post-GT, the subject continued to experience progression of disease, losing the ability to walk and speak followed by loss of hand, trunk, and head control. SAEs of spasticity and motor impairment were reported at approximately 5 months following treatment; events of motor impairment, spasticity, and dysphagia were reported as serious. The outcome of dysphagia was reported as fatal due to the inability to feed; the parents declined placement of a PEG feeding tube. Approximately 8 months after receiving treatment, the subject died. The investigator considered the events of motor dysfunction, muscle spasticity, and dysphagia as unrelated to Libmeldy-f and the death to be associated with disease progression.

One patient in the CUP programme (Patient 27) died after treatment with Libmeldy on Day 415 (13.8 months). The patient was presymptomatic (EJ variant) at the time of treatment and remained asymptomatic at the time of experiencing an ischemic cerebral infarction (left hemisphere cerebral ischemic stroke) on Day 414 that led to death (Grade 5) on Day 415. Routine follow-up for the patient performed at the Year 1 study visit prior to the SAE of ischemic cerebral infarction, showed that the patient was clinically well. The patient had no known predisposing risk factors for a thrombotic event, no vascular/endothelial complications after treatment, and no known history of trauma in the days leading up to the event. The cause of the event was unknown, but the investigator assessed the event of ischemic cerebral infarction as not related to Libmeldy, stating there was not sufficient information to establish a causal relationship between the event and gene therapy. The family declined post-mortem examination. The site continued to investigate the event in depth to define the exact nature, cause and pathophysiology of this acute event. The integration site analyses performed up to the latest follow-up by SR-TIGET showed highly polyclonal vector integration and did not reveal signs of clonal expansion or clonal dominance.

Adverse events of special interest

The applicant marks the following adverse events as significant and discusses them in more in detail: renal tubular acidosis / metabolic acidosis, hepatobiliary disorders, elevations in IgE and elevations in Ferritins. Therefore, these adverse events are grouped together by the assessors as “adverse events of special interest” and are discussed below.

Renal tubular acidosis/metabolic acidosis

It has been suggested that patients with MLD may develop an underlying proximal (Type 2) renal tubular acidosis due to sulfatide accumulation in the renal tubules. These patients may be at risk of metabolic acidosis in various acute clinical conditions such as infection.

Events of renal tubular acidosis and metabolic acidosis were defined based on venous blood gas parameters (blood pH and venous bicarbonate), urinary pH, and whether the acidotic event had occurred in combination with another clinical procedure (e.g., conditioning or general anaesthesia) or an acute clinical condition such an infection.

In the Integrated Safety Set, renal tubular acidosis or metabolic acidosis was reported prior to treatment with Libmeldy in 16 subjects (55%). In total (including before and after treatment with OTL-200-f), 19 subjects (66%) have presented with events of renal tubular acidosis or metabolic acidosis.

Renal tubular acidosis was reported in 8 subjects in the Pre-treatment phase, 4 subjects during the Treatment phase, and 2 subjects in the 3-month post-GT phase. The events occurring post-GT were considered to be related to the underlying disease and not to OTL-200-f.

Metabolic acidosis was reported in 3 subjects in the Pre-treatment phase, 4 subjects during the Treatment phase, 2 subjects in the 3-month post-GT phase, 1 subject in the Short-term phase, and 1 subject in the Long-term phase. The events occurring post-GT were considered to be related to the underlying disease and acute clinical condition at the time of the event.

Two subjects who experienced AEs of renal tubular acidosis prior to the treatment subsequently experienced SAEs of metabolic acidosis. In one of these cases, the SAE of metabolic acidosis was temporally associated with concurrent febrile neutropenia and mucositis. In the other case, the event of metabolic acidosis was reported with a concurrent event of upper airways infection. Both events resolved following treatment of the event and concurrent medical condition and were considered related to the underlying disease.

There were no significant findings indicating that the treatment or concomitant medications employed in gene therapy play a critical role in the exacerbation of renal impairments.

Hepatobiliary Disorders

Patients with MLD are known to be at increased risk of developing gallbladder abnormalities, including wall thickening and polyps, compared with patients with other lysosomal storage disorders or healthy patients. The deposition of accumulated sulfatide in visceral tissue has been implicated in these findings; the risk of gallbladder polyps evolving into carcinoma has been reported in MLD patients (Agarwal, 2013, Kim, 2017).

In the Integrated Safety Set, mild, non-serious gallbladder enlargement was reported in 22 subjects during the Pre-treatment phase. None of the subjects treated with Libmeldy presented with hepatic impairment prior to treatment.

During the Follow-up phase, 16 subjects experienced hepatobiliary AEs that included newly reported events of gallbladder enlargement (3 subjects); gallbladder polyps were reported in 4 subjects with pre-existing events of gallbladder enlargement. In 2 subjects, a cholecystectomy was performed due to findings of polyps >5 mm identified by ultrasound scan. Cholecystectomies were performed in consideration of the reported risk of gallbladder polyps evolving into carcinoma. In both cases, the polyps were reported as SAEs.

After excluding event terms related to the gallbladder, 11 subjects (38%) in the Integrated Safety Set had events in the hepatobiliary disorders SOC. These events included veno-occlusive liver disease, drug-induced liver injury, hepatomegaly, and hypertransaminasemia. Specifically, 3 patients in the CUP/HE programmes experienced veno-occlusive liver disease. Two subjects experienced hypertransaminasemia (one Grade 1 and one Grade 2). Two subjects also experienced gallbladder enlargement and acute cholecystitis, n=1 each.

It is also important to highlight that hepatic veno-occlusive disease and hepatomegaly are known safety concerns related to busulfan conditioning (Ciurea, 2009) and reported as ‘very common’ adverse reactions in Section 4.8 of the SmPC of the product which also reports that “Grade 3 elevated transaminases were reported in 24% of patients” (Busilvex Summary of Product Characteristics, 2017).

Elevations in Ig E

Increases in Ig E are typically associated with inflammatory responses. In the Integrated Safety Set, increased Ig E was reported in 4 subjects (14%) before treatment with OTL-200-f and in 13 subjects (45%) during the Follow-up post-GT phase. None of the AEs were serious, and the investigator assessed the events as unrelated to Libmeldy-f.

In Study 205756, 2 subjects experienced increased Ig E AEs; both events occurred prior to treatment with Libmeldy-c.

Elevations in Ferritin

Ferritin is an acute phase protein and can be increased secondary to several reasons, including inflammation, infection, or recent blood transfusion.

In the Integrated Safety Set, 6 subjects (21%) had AEs of serum ferritin increased; all were reported in the 3-month post-GT phase. The events were likely related to repeated transfusions during this time period. None of the AEs were serious, and the investigator assessed the events as unrelated to Libmeldy-f.

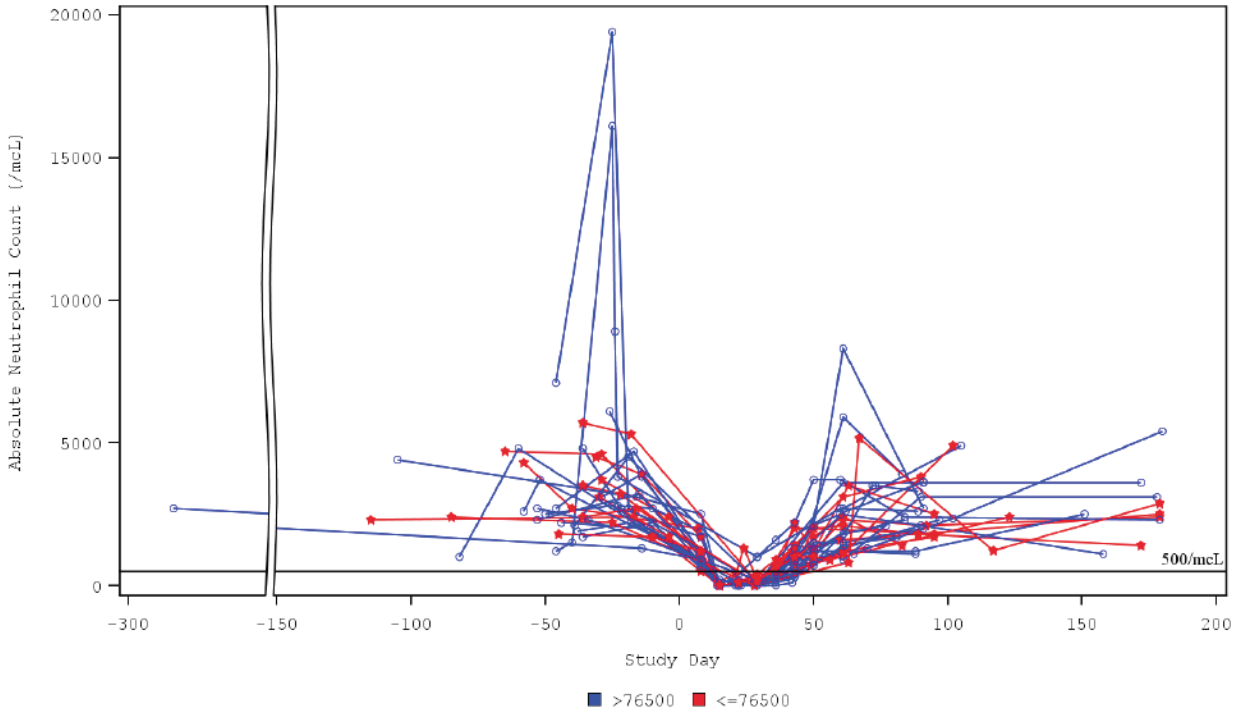
In Study 205756 of Libmeldy-c, there were no AEs of increased ferritin reported.

Laboratory findings

Haematology

Two subjects had a high ANC prior to treatment with Libmeldy (Figure 19) and all subjects in the Integrated Safety Set experienced severe neutropenia (ANC <500/mcL). All subjects, except 2, experienced absolute aplasia (ANC=0) following busulfan conditioning. ANC recovered over time (Figure 19), and no subject had an ANC <500/mcL at Day 60. The geometric mean (95% CI) number of days with an ANC of 0 was 4.6 days (n=27, 1.81 days, 11.50 days) and with an ANC <500 mcL was 27.6 days (25.06 days, 30.36 days).

Figure 19: Subject Profile of Absolute Neutrophil Count by Busulfan Area Under the Curve (Integrated Safety Set)



Source:

Note: Because the cumulative area under the curve in 2 subjects who received a sub-myeloablative conditioning regimen was outside the target range, data were analysed by total area under the curve rather than by conditioning regimen.

Five subjects from the Integrated safety set received G-CSF after conditioning and the timing in relation to gene therapy is outlined in Table 17. Three of the five subjects received the MAC conditioning regimen and two received the SMAC conditioning regimen. Four subjects treated under Study 201222 and EAPs (Patient 11,9,21,25) received G-CSF due to prolonged neutropenia (defined as neutropenia beyond Day +45 post-treatment) in absence of any concomitant acute illness/infection. None of those patients met the per-protocol criteria for engraftment failure. Two out of the five subjects required a single dose of G-CSF. One subject (Patient 21) experienced a nonserious AE of Grade 3 febrile neutropenia at Day +25, which was treated with support from G-CSF once a day from Day +25 to Day +41. On Day +44, G-CSF was restarted in response to a neutrophil count <0.5 x 10^9/L and administered as required to keep the neutrophils counts above 500/mmc until Day +52. On Day +118, the patient received a single dose of G-CSF due to transient neutropenia (ANC=560/mmc).

Table 17. List of subjects who received G-CSF post-treatment with Libmeldy

Subject ID	Timing post-gene therapy	Conditioning regimen
Patient 11	Day 46[1]	MAC
Patient 9	Day 46-48[2]	MAC
Patient 21	Day 25- Day +41 (once a day) Day 44, 46, 49-52 (once a day) Day 118[3]	MAC

Patient 22	Day 43, 45-46, 50, 55[4]	SMAC
Patient 25	Day 46[5]	SMAC

[1] G-CSF dose administered: 90 µg
[2] C-CSF dose administered: 40 µg /day
[3] G-CSF dose administered: 50 µg /day
[4] G-CSF dose administered: 40 µg /day
[5] G-CSF dose administered: 60 µg

Haematocrit, hemoglobin, leukocytes, and platelets followed a similar pattern with an initial decrease after busulfan condition followed by a recovery to pre-treatment levels by Day 60.

The median number of days to platelet engraftment was 41 (range: 14 to 109 days) with Libmeldy treatment. All patients treated with Libmeldy received transfusion support with platelets and these infusions were considered part of the standard of care/prophylaxis for these subjects. In addition, platelet transfusions were given also for the treatment of adverse events (1 event of thrombocytopenia, 1 event of febrile neutropenia, and 3 events of epistaxis (in 2 patients)). There was one patient who experienced thrombocytopenia leading to clinical sequelae. This event was seen in one subject during the 3-months post-GT phase in a complex post-transplant course, with SAEs of thrombocytopenia (Day 10), prolonged anaemia (Day 15), VOD (Day 19) and transplant-associated microangiopathy (TA-TMA) captured as atypical haemolytic uremic syndrome (Day 41). All the patients in the integrated safety set received transfusion support. Most of these transfusions were received during the peri-transplant period and mainly within the three months post gene therapy (≤100 days). One patient had a blood transfusion after 100 days post-GT (Day 382) after a decrease in haemoglobin concurrent to aspiration pneumonia. None of the 29 subjects in the integrated safety set received erythropoietin.

Nearly all subjects experienced low neutrophils and low platelets of potential clinical importance during follow-up (100% and 93%, respectively) (Table 18). No subjects experienced low hemoglobin, leukocytes, or lymphocytes of potential clinical importance after Day 60 or low platelets of potential clinical importance after Month 3. Subjects received routine standard of care procedures during treatment and hospitalisation, which includes transfusions to prevent severe anemia.

Table 18: Haematology Data of Potential Clinical Importance at Any Time during Follow-up (Integrated Safety Set)

Parameter, n (%)	Late Infantile (N=16)	Early Juvenile (N=13)	Total (N=29)
Hemoglobin Low	3 (19)	0	3 (10)
Leukocytes Low	6 (38)	11 (85)	17 (59)
Lymphocytes Low	1 (6)	1 (8)	2 (7)
Neutrophils Low	15 (94)	13 (100)	28 (97)
Platelets Low	15 (94)	12 (92)	27 (93)

In Study 205756 of Libmeldy-c, transient haematological (e.g. neutrophils, erythrocytes, and platelets) decreases were observed after the conditioning phase in all subjects. After busulfan conditioning, all subjects experienced severe neutropenia (ANC <500/µL). None of the subjects had an ANC <500/µL at Day 60. The number of days with an ANC <500/µL ranged from 36-41 days, and the number of days with an ANC of 0

ranged from 15-29 days. The number of days with an ANC <500/µL was in mean 39.2, ranged from 36-41 days, and the number of days with an ANC of 0 ranged from 15-29 days (in mean 20.7 days).

Chemistry

In the Integrated Safety Set, most clinical chemistry values remained within the normal laboratory ranges (Table 2.7.4.3.16). Few subjects experienced changes in chemistry values of potential clinical importance, and these events all resolved in a short period of time (Table 2.7.4.3.17).

ALT and AST increased after conditioning and then gradually returned to the normal range (Figure 20 and Figure 21). Alkaline phosphatase, bilirubin, and Gamma Glutamyl Transferase levels generally stayed within the normal range (Figure 2.7.4.3.18).

Figure 20: Box Plot of Alanine Aminotransferase Relative to the Upper Limit of Normal, by Visit (Integrated Safety Set)

Figure 21: Box Plot of Aspartate Aminotransferase Relative to the Upper Limit of Normal, by Visit (Integrated Safety Set)

For one patient (Patient 18) increases in ALT and AST levels to grade 3 were reported at Day -3 with a mild increase in GGT. Thereafter the levels decreased. Abdominal echo-scans were normal and the increase in liver enzymes was considered probably related by the investigator to conditioning with a possible contribution of amoxicillin/clavulanic acid and ceftriaxone. During the three months post-GT phase, ALT and AST levels remained high, with both ongoing as grade 3 AEs. On Day 4, a new peak was noted and again abdominal echo-scans were normal. The liver enzyme increases were considered to be a continuation of the previous events. At Day 14 again high ALT and AST values were reported with elevations in bilirubin and GGT at Day 15. At that point, Hy's Law criteria were met. To exclude hepatic veno-occlusive disease, an echo-scan of the abdomen was performed and showed a mild peritoneal liquid accumulation without any evidence of alteration of blood flux at the sovra-hepatic veins. The investigator stated that the increased levels of bilirubin could also be subsequent to haemolysis related to a blood transfusion performed the previous day. Oral treatment with ursodeoxycholic acid for prophylaxis started on Day 15 and stopped on Day 17 because of rapid decrease of liver damage indexes and bilirubin. Hy’s law criteria were no longer met and the liver enzymes progressively decreased. This was deemed a possible late effect of the conditioning by the investigator and according to the applicant, the contribution of amphotericin B cannot be excluded. The increase in GGT resolved on Day 60, increase in ALT on Day 186, and increase in AST on Day 277. The investigator highlighted the relevance of multiple antibiotic therapy given for the febrile neutropenia and device related infection events and stated that the subsequent increases in liver enzymes may have resulted from an initial liver insult provoked by busulfan conditioning being possibly exacerbated by the concomitant infectious complications which have required administration of multiple drugs. For another patient Patient 13), ALT increased (grade 1) and AST increased (grade 1) were reported from Day 39 to Day 75, for which no action was taken. No additional testing or specific radiological investigation was deemed necessary by the investigator to further characterise these transaminase increases. Both AEs resolved approximately 1 month

later, with no corrective treatment and the subject did not experience any additional events of increased ALT/AST in the subsequent phases. The investigator stated that the concomitant medications (including amphotericin B and fluconazole), the recent occurrence of febrile neutropenia and busulfan conditioning could have all have played a role in the development of liver injury. In Study 205756, there have been no laboratory parameters reported to be of severity NCI CTC Grade ≥3 during the 3-month post-treatment phase.

Coagulation

Outside the normal range were reported for activated PTT (above the ref. range in 10/28 pts [36%]), for D-dimer (above the ref. range in 17/28 pts [61%]), and Antithrombin III decreased in one patient after 3 months of Libmeldy-c.

Pituitary-thyroid axis and Glucose

Thyrotropine (TRH) was reported > ref. range high in 9/28 patients (32%), increased in 3 LI at baseline; thyroxine free (FT4) was > ref. range high in 2/8 patients analysed and triiodothyroxine free (FT3) was > ref range high in 10/28 patients (36%).

Protein electrophoresis

In the Integrated Safety Set, the applicant concludes that most protein electrophoresis values remained within the normal laboratory ranges over time. No notable changes in protein electrophoresis were observed during the study.

Replication competent lentivirus

There were no confirmed positive results for RCL.

Malignancies and abnormal clonal proliferation

No malignancies were reported after treatment with OTL-200-f or OTL-200-c.

Evidence of abnormal haematopoietic clonal proliferation was assessed by clinical and laboratory surveillance and BM examination. There was no evidence of clonal expansion as assessed by BM and PB lymphocytes karyotype, morphological analyses in the BM, immune phenotyping, and T cell receptor repertoire.

Vital signs

In the Integrated Safety Set, the applicant concludes that the vital signs were generally within age-appropriate normal ranges. Changes in vital signs were mostly considered not clinically relevant. No significant changes were observed in the vital signs after treatment with Libmeldy-f. In Study 205756 of Libmeldy-c, some vital sign values were reported as transiently out of range according to the applicant.

Safety in special populations

Adverse events by MLD variant

AEs were analysed by MLD early-onset variant, either LI or EJ. MLD variant was determined per study protocol but generally took into account the age at onset of symptoms or age at onset of symptoms of the index case in the family, genotype, and the presence of documented peripheral neuropathy at electroneurogram recordings.

Numerical differences were observed between the AEs in subjects with LI MLD and those with EJ MLD (see Table 19. A higher percentage of subjects with EJ MLD (85%) than subjects with LI MLD (38%) experienced AEs during the Treatment phase. Subjects with LI MLD experienced fewer SAEs during the Follow-up phase compared with subjects with EJ MLD (63% vs 77%).

Table 19 Adverse Events by MLD variant

Category	Late Infantile (N=16)			Early Juvenile (N=13)		
	n	%	# of Events	n	%	# of Events
Pre-treatment						
AEs	16	100	46	13	100	33
SAEs	2	13	2	0	0	0
Treatment Phase						
AEs	6	38	8	11	85	15
Treatment-related AEs	0	0	0	0	0	0
AEs Leading to Withdrawal	0	0	0	0	0	0
SAEs	0	0	0	0	0	0
Deaths	0	0	0	0	0	0
Treatment-related SAEs	0	0	0	0	0	0
AE by Grade						
Grade 1	4	25	4	6	46	6
Grade 2	2	13	2	1	8	1
Grade 3	2	13	2	6	46	8
Grade 4	0	0	0	0	0	0
Grade 5	0	0	0	0	0	0
Follow-up Post-GT Phase						
AEs	16	100	300	13	100	165
Treatment-related AEs	4	25	6	0	0	0
AEs Leading to Withdrawal	0	0	0	3	23	3
SAEs	10	63	26	10	77	16
Deaths	0	0	0	3	23	3
Treatment-related SAEs	0	0	0	0	0	0
AEs by Grade						
Grade 1	16	100	72	12	92	45
Grade 2	15	94	128	12	92	46
Grade 3	16	100	96	13	100	68
Grade 4	3	19	4	3	23	3
Grade 5	0	0	0	3	23	3

Adverse events by symptomatic status at time of treatment

AEs were analysed by symptomatic status, and the population was broadly divided into subjects who were pre-symptomatic and subjects who were symptomatic at the time of treatment. Pre-symptomatic status was defined per study protocol.

Subjects who were symptomatic at the time of GT experienced more AEs during all study phases (see Table 20).

Table 20 Adverse Events by Symptomatic Status at the Time of Gene Therapy (Integrated Safety Set)

Category	Pre-Symptomatic at GT (N=20)			Symptomatic at GT (N=9)		
	n	%	Number of Events	n	%	Number of Events
Pre-treatment						
AEs	20	100	55	9	100	24
SAEs	2	10	2	0	0	0
Treatment Phase						
AEs	9	45	12	8	89	11
Treatment-related AEs	0	0	0	0	0	0
AEs Leading to Withdrawal	0	0	0	0	0	0
SAEs	0	0	0	0	0	0
Deaths	0	0	0	0	0	0
Treatment-related SAEs	0	0	0	0	0	0
AE by Grade						
Grade 1	6	30	6	4	44	4
Grade 2	2	10	2	1	11	1
Grade 3	4	20	4	4	44	6
Grade 4	0	0	0	0	0	0
Grade 5	0	0	0	0	0	0
Follow-up Post-GT Phase						
AEs	20	100	321	9	100	144
Treatment-related AEs	4	20	6	0	0	0
AEs Leading to Withdrawal	1	5	1	2	22	2
SAEs	14	70	25	6	67	17
Deaths	1	5	1	2	22	2
Treatment-related SAEs	0	0	0	0	0	0
AEs by Grade						

Grade 1	19	95	83	9	100	34
Grade 2	19	95	138	8	89	36
Grade 3	20	100	96	9	100	68
Grade 4	2	10	3	4	44	4
Grade 5	1	5	1	2	22	2

Abbreviations: AE=adverse event; GT=gene therapy; SAE=serious adverse event

Events associated with MLD were more common in subjects who were symptomatic at the time of treatment than in subjects who were pre-symptomatic (see Table 21).

Table 21 Adverse Events Related to MLD by Symptomatic Status at the Time of Gene Therapy

Preferred Term, n (%)	Pre-Symptomatic (N=20)				Symptomatic (N=9)			
	3 Month Post-GT (N=20)	Short Term (N=20)	Long Term (N=9)	Total Follow-up Post-GT (N=20)	3 Month Post-GT (N=9)	Short Term (N=9)	Long Term (N=7)	Total Follow-up Post-GT (N=9)
Gait Disturbance	2 (10)	6 (30)	0	8 (40)	3 (33)	3 (33)	1 (14)	7 (78)
Motor Dysfunction	1 (5)	2 (10)	0	3 (15)	1 (11)	5 (56)	0	6 (67)
Muscle Spasticity	0	1 (5)	1 (11)	2 (10)	1 (11)	5 (56)	1 (14)	7 (78)
Aphasia	0	0	1 (11)	1 (5)	1 (11)	4 (44)	0	5 (56)
Ataxia	0	0	0	0	2 (22)	3 (33)	0	5 (56)
Dysarthria	0	1 (5)	0	1 (5)	1 (11)	3 (33)	0	4 (44)
Cognitive Disorder	0	0	1 (11)	1 (5)	0	3 (33)	0	3 (33)
Dysphagia	0	0	1 (11)	1 (5)	0	3 (33)	0	3 (33)
Renal tubular acidosis	0	0	0	0	2 (22)	0	0	2 (22)
Metabolic acidosis	1 (5)	1 (5)	0	2 (10)	1 (11)	0	1 (14)	2 (22)
Gallbladder Enlargement	0	0	1 (11)	1 (5)	1 (11)	1 (11)	0	2 (22)
Gallbladder Polyp	0	3 (15)	0	3 (15)	0	1 (11)	0	1 (11)
Seizure	0	1 (5)	0	1 (5)	0	0	1 (14)	1 (11)
Foot Deformity	0	0	0	0	0	1 (11)	0	1 (11)

Abbreviations: GT=gene therapy; MLD=metachromatic leukodystrophy

Adverse events by busulfan conditioning regimen

The percentages of subjects who experienced an adverse event within the first three months (≤100 days) following treatment (treatment, acute, 3-months post-GT phases) for the SMAC and MAC groups are shown in Table 22.

Table 22. Incidence of Adverse Events by Busulfan Regimen within three months **(≤100** days) post-GT (Integrated Safety Set)

Phase	MAC (N=16)		SMAC (N=13)	
	n (%) of subjects	# of events	n (%) of subjects	# of events
Treatment	10 (63%)	15	7 (54%)	8
Acute	1 (6%)	1	2 (15%)	2
3 months post-GT	16 (100%)	110	12 (92%)	71

Source: Table 2.7.3.2.1.3.a

Febrile neutropenia and stomatitis were more common in subjects who received a MAC regimen than in subjects who received a SMAC regimen (94% versus 62% and 63% versus 15%, respectively) (Table 22). Subjects were also more likely to have AEs of serum ferritin increased in the MAC group than in the SMAC group (38% versus 0%, respectively), which could be related to the need for transfusions after MAC. VOD occurred in 2 subjects (13%) who received a MAC regimen and 1 subject (8%) who received a SMAC regimen; aHUS occurred in 2 subjects (13%) who received a MAC regimen and did not occur in subjects who received a SMAC regimen (Table 2.7.4.2.1.36).

A small difference was observed between conditioning groups in the number of days with an ANC below 500/µL (26.9 days [95% CI 23.79-30.35 days] SMAC and 28.2 days [95% CI 24.12-32.92 days] MAC). There was also a small difference between conditioning groups in the number of days with an ANC of 0/µL (3.9 days [95% CI 0.82-18.20 days] SMAC and 5.2 days [95% CI 1.46-18.66 days] MAC). During the Treatment phase, metabolic acidosis was only reported in subjects treated with a MAC regimen. Additionally, during the 3 months post-treatment phase, metabolic acidosis was only reported in subjects who had received a SMAC regimen. At any time, hepatomegaly was reported in 4 out of 13 subjects receiving a SMAC regimen and 2 out of 16 subjects receiving a MAC regimen.

Table 23 Adverse Events in at Least 10% of Subjects Overall by Preferred Term, by Busulfan Regimen, Treatment Phase and 3-month Post-GT Phase (Integrated Safety Set)

Preferred Term	SMAC Regimen (N=13)			MAC Regimen (N=16)			Total (N=29)		
	n	%	#	n	%	#	n	%	#
Treatment Phase									
Metabolic Acidosis	0	0	0	4	25	4	4	14	4
Renal Tubular Acidosis	2	15	2	2	13	2	4	14	4
3-month Post-GT Phase									
Febrile Neutropenia	8	62	9	15	94	17	23	79	26
Device Related Infection	1	8	1	3	19	3	4	14	4
Stomatitis	2	15	2	10	63	10	12	41	12
Mucosal Inflammation	6	46	6	4	25	4	10	34	10
Blood IgE Increased	2	15	2	4	25	4	6	21	6
Rash Erythematous	4	31	4	2	13	3	6	21	7
Serum Ferritin Increased	0	0	0	6	38	6	6	21	6
Gait Disturbance	2	15	2	3	19	3	5	17	5
Neutropenia[a]	2	15	2	3	19	3	5	17	5
Clostridium Difficile Colitis	1	8	1	2	13	2	3	10	3
Hepatomegaly	2	15	2	1	6	1	3	10	3
Upper Respiratory Tract Infection	2	15	2	1	6	1	3	10	3
Venooclusive Liver Disease	1	8	1	2	13	2	3	10	3
Vomiting	1	8	2	2	13	5	3	10	7

[a] Prolonged neutropenia (neutropenia beyond Day 45 post-treatment)
Note: No adverse events in the Acute phase occurred in 10% or more of subjects.
Abbreviations: #=number of events; GT=gene therapy; IgE=immunoglobulin E; MAC=myeloablative conditioning; SMAC=sub-myeloablative conditioning

Differences in common AEs between groups based on total busulfan AUC generally followed the same pattern as differences based on conditioning regimen. As would be expected based on the safety profile of busulfan, stomatitis was more common in subjects with a total busulfan AUC >76500 µg*h/L than in subjects with a total busulfan AUC ≤76500 µg*h/L.(56% versus 18%, respectively) (Table 23). Subjects were also more likely to have AEs of serum ferritin increased with a total busulfan AUC >76500 µg*h/L than with a total

busulfan AUC ≤76500 µg*h/L (33% versus 0%, respectively). Otherwise, there were no meaningful differences noted in common AEs between conditioning groups based on total busulfan AUC.

A small difference was observed between conditioning groups by total busulfan AUC in the number of days with an ANC below 500/µL (26.0 days [95% CI 22.78-29.63 days] total busulfan AUC ≤76500 µg*h/L and 28.6 days [95% CI 24.89-32.89 days] total busulfan AUC >76500 µg*h/L).

Table 24 Adverse Events in at Least 10% of Subjects Overall by Preferred Term, by Busulfan Total Area Under the Curve, Treatment Phase, Acute Phase, and 3-month Post-GT Phase (Integrated Safety Set)

Preferred Term	**≤76500µg*h/L** (N=11)			**>76500µg*h/L** (N=18)			Total (N=29)		
	n	%	#	n	%	#	n	%	#
Treatment Phase									
Metabolic Acidosis	0	0	0	4	22	4	4	14	4
Renal Tubular Acidosis	2	18	2	2	11	2	4	14	4
3-month Post-GT Phase									
Febrile Neutropenia	8	73	9	15	83	17	23	79	26
Stomatitis	2	18	2	10	56	10	12	41	12
Mucosal Inflammation	4	45	5	5	28	5	10	34	10
Blood IgE Increased	1	9	1	5	28	5	6	21	6
Rash Erythematous	4	36	4	2	11	3	6	21	7
Serum Ferritin Increased	0	0	0	6	33	6	6	21	6
Gait Disturbance	1	9	1	4	22	4	5	17	5
Neutropenia[a]	2	18	2	3	17	3	5	17	5
Device-related Infection	1	9	1	3	17	3	4	14	4
Clostridium Difficile Colitis	1	9	1	2	11	2	3	10	3
Hepatomegaly	1	9	1	2	11	2	3	10	3
Upper Respiratory Tract Infection	1	9	1	2	11	2	3	10	3
Venooclusive Liver Disease	1	9	1	2	11	2	3	10	3
Vomiting	1	9	2	2	11	5	3	10	7

[a] Per protocol, neutropenia occurring within the first 3 months post-GT was only reported as an AE if National Cancer Institute Common Toxicity Criteria was at least Grade 3.
Note: No adverse events in the Acute phase occurred in 10% or more of subjects.

Abbreviations: #=number of events; AE=adverse event; GT=gene therapy; IgE=immunoglobulin E

Pregnancy & Lactation

The effect on breast-fed infants of administration of Libmeldy to their mothers has not been studied. The excretion of Libmeldy in milk has not been studied in humans or animals.

Age at the time of treatment for subjects in the Libmeldy programme ranged from 7 months to 11 years. There were no pregnancies reported.

Immunological events

AAA were detected in four of 29 subjects treated with the fresh formulation of Libmeldy.

Antibody titres in all 4 subjects were generally low and at the time of the data cut, all had resolved to negative test results, either spontaneously (n=1) or after 1 cycle of rituximab (n=3). In two cases (Patient 25 and 28), gait disturbance developed after the detection of AAAs.

For Study 201222 (where AAA were tested from 3 months post-treatment onwards), no subjects have tested positive for AAA and none of the treated subjects under EAPs tested positive for antibodies at the baseline visit.

Safety related to drug-drug interactions and other interactions

No formal drug-drug interaction studies were performed.

Discontinuation due to AES

Three subjects discontinued the study early due to a SAE of death.

Post marketing experience

N/A

2.8.1. Discussion on clinical safety

The applicant discussed the safety based on the integrated safety set. This set consisted of 20 subjects from the registrational study 201222 and 9 subjects from the expanded access programmes. The number of patients in the Libmeldy safety set is therefore limited. The known safety profile of autologous HSCT and busulfan conditioning will however support in the interpretation of the safety profile of Libmeldy treatment. Safety data from the ongoing study 205756 with the cryopreserved formulation was presented separately.

The median duration of follow up for the original MAA data cut is approximately 3 years, ranging from 1 to 7 years. For study 205756 with the cryopreserved formulation the follow up was 30 days, 3 months, 6 months and 1 year respectively. For the integrated safety set, the data cut-off was mid-to-end of year 2018. Additional safety data was provided by the applicant, with the new cut-off at end of 2019 for Study 20122 and March 2020 for the expanded access programme. In the updated safety data set (n=29) the median

duration of follow up was 4.5 years, ranging from 0.6 to 9 years. The new data cut for the cryopreserved study was end of 2019. Two additional subjects received Libmeldy-c as of the new data cut. The mean follow-up for study 205756 (n=6) is 0.7 range, range 0 to 1.5 years. Overall, the data of the updated safety data set appears to be similar to what was observed in the original MAA data cut.

For gene therapies with genome editing properties, the safety follow-up should be 15 years. To address this issue, the applicant intends to perform a post authorisation study called LongTERM-MLD. In this study, patients will be included from the clinical development programme as well as new patients treated with the commercial formulation in accordance with an agreed protocol.

As first part of Libmeldy treatment, patients will undergo BM harvest or mobilised PB apheresis and the safety profile of these procedures is described in section 4.8 of the SmPC. There are uncertainties regarding the cell collection procedures for Libmeldy treatment. The recommended amount of harvested BM in young children is no more than 20 ml/kg (Transfusion and apheresis Science 2018, 57, 323-330, Blood 2012, 119, 2935-2942). According to the clinical trial protocol the BM volume collected was set at 20 to 25 mL/kg of donor weight. However, the amount of collected BM was adjusted for each subject in order to ensure that the desired target was reached. The median was 36.20 mL/kg (range: 17.64 to 56.60) in the pivotal trial with fresh formulation. The applicant acknowledges that the standard volume aimed for collection of a bone marrow sample in the allogeneic setting is ≤ 20 ml/kg. Taking into account the possible cell loss during transport and manufacturing process, as well as the correlation between cell-dose and engraftment in haematopoietic transplant medicine, in the Libmeldy studies higher volumes of BM were harvested for the manufacture of Libmeldy in some cases (ranging from 15.09 to 56.40 ml/kg patient body weight) depending on specific patient conditions and based upon an individualized risk-benefit assessment by the clinicians. The safety profile of BM harvest was consistent with the known safety and tolerability of the procedure. Therefore, collection of larger quantities than the standard volume used in allogenic setting can be accepted if this is needed to collect sufficient cell numbers for production of Libmeldy. Importantly, the SmPC adequately describes in the cellular source of choice and quantity of $CD34^+$ cells required in section 4.2 and the safety profile of the BM harvest is described in section 4.8. In addition, the applicant will include recommendations regarding the BM harvest as part of the site qualification process.

The median volume of bone marrow harvested for the cryopreserved product was within the range of the bone marrow volume harvest for the manufacturing of the fresh product. A single leukapheresis procedure encompassing a maximum of two cell collection cycles (performed in up to 2 consecutive days) following mobilisation with G-CSF and Plerixafor, provided a sufficient number of CD34+ for both DP manufacture and Back up cells, in all patients treated with either formulation of Libmeldy.

Adverse events

The applicant has allocated the adverse events per pre-specified treatment phase: pre-treatment, treatment (first day conditioning – GT infusion), acute phase (up to 48 hours after GT), 3-month post GT phase (until day 100), short term phase (day 101 – 1098) and long-term phase (> 1098). In any given phase, only those adverse events that started during that phase were reported. Adverse events reported in previous phase(s) and were ongoing at the start of subsequent phase(s) were not reported in those subsequent phase(s). Thus, the data convey no information on the duration of the adverse events observed. Information was provided by the applicant on the median duration of common adverse events and adverse events rated grade 3 or higher. Adverse events related to MLD appear to be the most persistent events.

Overall, the number of days in hospital post-treatment for all patients treated with Libmeldy in the clinical development programme ranged from 37 to 94 days (approximately 4 to 12 weeks, as stated in the SmPC), in line with what is observed in the post-transplant course after a myeloablative conditioning regimen and in other gene therapy clinical trials. Two patients (Patient 14 and 21) stayed in hospital 82 days and 94 days post-treatment respectively, due to some complications considered unrelated to Libmeldy.

Adverse events were reported with a high frequency. The applicant assigned the common adverse events observed into 3 general categories: adverse reactions related to Libmeldy, adverse reactions related to busulfan conditioning and adverse reactions related to MLD. This will be discussed in further detail below.

In the pre-treatment phase, the most common adverse event is gallbladder enlargement (76%). This may be due to the underlying disease pathology, as no intervention has taken place yet.

In the acute phase (≤ 48 hours after gene therapy) the only event reported was hepatomegaly (3%) which was likely attributable to busulfan conditioning.

In the 3-month post GT febrile neutropenia (79%), stomatitis (41%) and mucosal inflammation (34%) are most commonly reported. These events most likely are due to the busulfan conditioning regimen that subjects received prior to GT. An exception is the occurrence of rash erythematous (21%). A relationship with Libmeldy is considered unlikely, as the events did not occur immediately after infusion and subjects were taking medication concomitantly which are also known to cause rash.

In the short-term follow up (100-1098 days post gene therapy), several adverse events are reported that appear suggestive of MLD disease progression, i.e. gait disturbance (31%), motor dysfunction (24%), muscle spasticity (31%) ataxia (10%), aphasia (14%), dysarthria (14%) and dysphagia (10%).

In the long term follow up (> 1098 days i.e. > 3 years post gene therapy), the common adverse events were upper respiratory tract infection (31%), vitamin D decreased (25%) and pyrexia (19%). The most common AEs during the long-term phase were related to infections and infestations (50% of subjects), predominantly of the upper respiratory tract (n=5, 31%). Most patients in the LI group were within the pre-school age (4-6 years) and the rate of upper respiratory tract infections (URTIs) should be considered “normal” as observed within certain frequencies (up to 8 episodes of URTIs per year in the pediatric population) during pre-school age. There is no evidence suggesting a relationship between the frequency of infections during the long-term phase and immunological reconstitution. It is however recommended to follow-up infectious events and immunological parameters as part of the routine follow-up in ongoing studies.

Adverse reactions related to conditioning regimen

AEs such as (febrile) neutropenia, other cytopenias, mucosal inflammation, stomatitis, venoocclusive disease, infections, and increases in liver enzymes are known to be associated with busulfan and it is agreed with the applicant that AE observed in these categories likely are due to the myeloablative conditioning. All grade ≥3 infections occurring within the first 3 months resolved and none was reported as a SAE. Based on the duration of follow-up, long-term toxicity of the conditioning regimen cannot be determined, such as the occurrence of second primary malignancies.

Overall, the safety profile of the busulfan conditioning regimen is as expected and substantial but manageable.

Adverse reactions related to Libmeldy

The only AE that were related to Libmeldy treatment were anti ARSA antibody test positive in 4 (14%) subjects. The clinical consequences of positive anti ARSA antibodies will be discussed below in the section "Immunological events".

Adverse reactions related to MLD

Symptoms of MLD were only reported if clinically significant and had an NCI Grade ≥ 3. In the integrated safety set, several subjects experienced adverse events which were attributed to MLD. The most frequent were gait disturbance (15 subjects), motor dysfunction (9 subjects), muscle spasticity (9 subjects) and aphasia (6 subjects). It is possible that other more subtle events are misclassified as not attributed to MLD or as they were not classified as grade 3 are higher. In the proposed PAES study, symptoms of MLD will be recorded regardless of severity.

Serious adverse events

In the 3-month post GT phase, common adverse events graded 3 or higher were related to the busulfan conditioning (e.g. febrile neutropenia, stomatitis, and neutropenia). In the follow up serious events related to MLD were more commonly reported.

There were two SAEs, both cases concerned atypical haemolytic uremic syndrome (aHUS). For one subject (Patient 21), it appears that prior events (e.g. positive anti CFH antibodies) and a specific genetic mutation made the subject more susceptible to aHUS. For the other subject (Patient 28), the requested narrative was provided and this case was determined to be transplant-associated thrombotic microangiopathy (TA-TMA), instead of aHUS. It was labelled as aHUS as TA-TMA does not exist in the MeDRA database. A discussion was provided on the relationship between MLD, aHUS and Libmeldy gene therapy for three cases Patient 21 (aHUS), Patient 28(TA-TMA) and Patient 22, who is the monozygous twin of Patient 21. In two cases (Patient 21 and 28), the events appear to have been caused by an interaction between predisposing risk factors such as age, the underlying MLD and in the case of Patient 21 genetic predisposition, and endothelial injury caused by the busulfan conditioning regime. Moreover, due to the development of aHUS in Patient 21 and the shared genetic predisposition, steps were taken in the gene therapy procedure for his twin to minimise the risk for aHUS development. Due to these countermeasures, the twin did not develop aHUS or TA-TMA. Based on the experience of TMA in these three cases, the applicant recommends prophylactic treatment with ursodeoxycholic acid and/or defibrotide prior to Libmeldy treatment in the SmPC, which is agreed.

Deaths

Three deaths occurred in the clinical development programme of Libmeldy: two in the registrational study and one in the expanded access programme. Two subjects (Patient 19 and 20) died due to MLD progression (dysphagia with fatal outcome). These are considered treatment failures.

One subject (Patient 27) died due to an ischemic cerebral infarction. A relationship between the ischemic event and gene therapy appears unlikely as the event took place approximately a year post gene therapy. Notably, the subject was responsive to treatment. Further information related to the case suggests that an infection is likely the cause of the event, however it was not possible to determine whether this had a bacterial or viral origin.

Adverse events of special interest

The applicant marks the following adverse events as significant and discusses them in more in detail: renal tubular acidosis / metabolic acidosis, hepatobiliary disorders, elevations in IgE and elevations in Ferritins.

Therefore, these adverse events are grouped together by the assessors as “adverse events of special interest” and are discussed below.

Renal tubular acidosis/metabolic acidosis. There were 8 cases of renal tubular acidosis in the pre-treatment phase of the integrated safety set. Given the high number of subjects who had renal tubular acidosis prior to Libmeldy treatment, the applicant’s interpretation that these events are likely to be due to the underlying arylsulfatase deficiency is supported. Metabolic acidosis was reported by a total of 11 subjects across the treatment phases. The Lorioli (2015) article cited by the applicant discusses part of the current patient cohort where subjects with renal tubular acidosis prior to treatment experienced metabolic acidosis coinciding with other major events such as surgery, sepsis or neutropenia. A possible mechanism coined by the authors is that metabolic stress could exacerbate the dysfunction already present, leading to an acute increase in the loss of bicarbonate and a rapid worsening of metabolic acidosis. Though, as noted by the applicant, there were only two subjects in the programme which had renal tubular acidosis at baseline. Risks associated with the gene therapy can also exacerbate independently the risk of developing metabolic acidosis. Moreover, there were also subjects in the clinical development programme who had metabolic acidosis prior to the gene therapy procedures.

Nevertheless, subjects with MLD eligible for Libmeldy therapy have an increased risk for metabolic acidosis due to their underlying condition and that the gene therapy is associated with techniques and adverse events that can exacerbate metabolic stress. To account for this, the applicant has included metabolic acidosis as a common side effect in section 4.8 and an additional warning in Section 4.4 of the SmPC is proposed. This is considered sufficient. Pending new information from the ongoing long-term safety evaluation the warning may need further amendment.

Hepatobiliary disorders. Non serious gallbladder enlargement was reported in 22 subjects of the integrated safety set during the pre-treatment phase. Gallbladder polyps were reported in 4 subjects with a pre-existing gallbladder enlargement. In two subjects a cholecystectomy was performed to remove the gallbladder polyps. The applicant’s interpretation that enlarged gallbladder/gallbladder polyps are related to MLD and not a risk of treatment is supported. Diagnostic imaging evaluations were performed at baseline to evaluate any structural abnormality related to gallbladder, liver or kidney. But gallbladder abnormalities or metabolic acidosis detected at baseline were not sufficient to exclude any subject from the trial. Therefore, the treating physician should confirm that the hepatic and renal functions are appropriate before the start of myeloablative conditioning, as is reported in section 4.2 of the SmPC.

Elevations in IgE, glucose and ferritins. IgE, glucose, ferritins and protein electrophoresis were measured as part of routine surveillance in accordance with the San Raffaele hospital guideline. There are no safety signs from the available safety data on IgE, glucose, ferritins and protein electrophoresis that would warrant consequences for the SmPC.

Laboratory measurements & vital signs

Haematology. After the busulfan conditioning, all patients had ANC <500/µL and most (93%) had absolute aplasia (ANC 0) which is expected. The geometric mean (95% CI) number of days with ANC of 0 was 4.6 days and the mean number of days with ANC <500 µL was 27.6 days. In total 5 patients received G-CSF (3 after MAC and 2 after SMAC). Prolonged neutropenia was the reason for G-CSF treatment in 4 patients and febrile neutropenia in 1. Furthermore, it seems that neutrophil engraftment after busulfan might be slower for MLD patients treated with Libmeldy compared to what is described in the SmPC of busulfan and Zynteglo. According to the applicant this is likely due to the pre-specified use of G-CSF after gene therapy. In the Libmeldy protocol, G-CSF treatment was allowed at day 45 post-treatment in case of prolonged neutropenia.

As described above, 4/29 patients (14%) received G-CSF due to prolonged neutropenia as per protocol in the absence of any concomitant acute illness/infection. According to the SmPC of Zynteglo, a total of 28.6% of patients received G-CSF within 21 days after Zynteglo infusion.

Also thrombocytopenia was, not unexpected, frequently observed after busulfan conditioning. The median number of days to platelet engraftment was 41 (range: 14 to 109 days) with Libmeldy treatment, which is longer than expected and comparable to what was observed for Zynteglo for which delayed platelet engraftment was noted as identified risk. All patients treated with Libmeldy received transfusion support with platelets and these infusions were considered part of the standard of care/prophylaxis for these subjects. In addition, platelet transfusions were given also for the treatment of adverse events (thrombocytopenia, febrile neutropenia, and epistaxis). There was one patient who experienced thrombocytopenia leading to clinical sequelae. This event was seen in MLD-HE01 during the 3-months post-GT phase in a complex post-transplant course, with SAEs of thrombocytopenia, prolonged anaemia, VOD and TA-TMA captured as atypical haemolytic uremic syndrome (Day 41). Although it is reassuring that no serious bleedings occurred, the information regarding the delayed platelet engraftment is included as a warning in the SmPC and as an important identified risk. According to the RMP, the important identified safety concern of delayed platelet engraftment will be further characterised after the treatment of new patients in the context of clinical trials open to recruitment (Study 205756 and Study Libmeldy-07), and the treatment of patients after Libmeldy marketing authorisation (MA) approval and followed up as part of LongTERM-MLD study.

Other myelosuppressive effects, such as anaemia, lymphopenia, and lymphocytopenia occurred as expected. All the patients in the integrated safety set received transfusion support. Most of these transfusions were received during the peri-transplant period and mainly within the three months post gene therapy (≤100 days). One patient had a blood transfusion after 100 days post-GT (Day 382) after a decrease in haemoglobin concurrent to aspiration pneumonia. None of the 29 subjects in the integrated safety set received erythropoietin. Moreover, PTT value was reported above normal reference range in 11/28 patients (39%). Since this ADR does not appear in the busulfan EU PI, it cannot be reflected in PI as associated to busulfan. It is also agreed that this ADR should not be mentioned outside of the ADR table in section 4.8 because a reasonable possibility of a causal relationship between the medicinal product and the adverse event would be needed, and this would not be the case for Libmeldy. Furthermore, the ADR cannot be confirmed at present as related to busulfan as it is not mentioned in the EU PI.

Chemistry. Increased liver enzymes were generally observed in the beginning of the treatment but returned to normal values around Month 3. There was one case meeting Hy's law criteria (MLD17) which is probably explained by the use of busulfan and concomitant medication and not by Libmeldy. The increases in liver enzymes and hepatomegaly are known for busulfan and are described in the SmPC. There are currently no hepatic safety concerns associated with the administration of Libmeldy.

Thyroid status. Accurate monitoring of the thyroid status in MLD patients is of pivotal importance because of the well-known effect of thyroid hormones on brain and body development, particularly in very young children. In general, increases in TSH, FT4 and FT3 were observed following treatment with Libmeldy, however plasma levels of these hormones were not specified. Plasma levels of thyroid parameters (TSH, FT3 and FT4) were provided. In general, increases in TSH, FT4 and FT3 were observed over the course of the study, but the variations in timings suggest that these increases should be unrelated to one another, as well as to gene therapy or associated procedures. However, during the study, clinicians did not detect any pattern of signs or symptoms in any subject to raise clinical concern for a thyroid disorder. It is appropriate to monitor thyroid function prior to treatment and shortly after treatment due to the risk of thyroid disorders

being masked by a critical illness or induced by concomitant medication given to children whilst in intensive care as is reflected in section 4.4 of the SmPC.

Lentivirus infectious particles. While residual LVV infectious particles are present in the drug product, the risk for secondary infection in patients due to these residual infectious virus particles is considered negligent. Furthermore, testing for replication competent lentivirus did not result in any confirmed positive sample for RCL.

Malignancies and abnormal clonal proliferation. No malignancies were reported in the Follow-up after treatment with Libmeldy-f or Libmeldy-c. In addition, there was no sign of clonal dominance or clonal proliferation from insertion site analyses. Nevertheless, this important potential risk will have to be followed post-approval as FU is too short to exclude this risk.

Special populations

Adverse events by MLD variant (LI, EJ). Upon request a table of the most common adverse events per MLD variant was provided per SOC and by preferred term. In general, a higher percentage of subjects with EJ MLD (85%) compared to patients with LI MLD (38%) experienced AEs during the treatment phase, and SAEs during the follow-up phase (77% of EJ vs. 63% of LI). In the LI group the most frequent AEs were infections (100% of patients), in particular upper respiratory tract infections (56%), or device related infection (44%). In the EJ group, febrile neutropenia was the most frequent AE (92% of patients vs. 69% of LI), whereas infections were observed in 77% of patients. With respect to the busulfan conditioning regimen, 20 patients were treated with MAC (14 EJ and 6 LI) and 13 subjects were treated with SMAC (12 LI and 1 EJ). The higher frequency of febrile neutropenia in EJ vs LI patients was likely to be attributed to prolonged neutropenia due to higher exposure to a conditioning regimen, as a common adverse event related to busulfan. Moreover, most of the subjects who received the MAC regimen were in the EJ cohort compared to the LI cohort (69% vs 44%). However, none of the events of febrile neutropenia were clinically significant and all resolved in a few days (median 3.5 days, range 2-5 days).

Considering the role of myeloablative conditioning level in the report of specific SAEs, percentages of subjects who experienced moderate events (63% in MAC versus 69% in SMAC), severe events (94% in MAC versus 92% in SMAC) and life-threatening events (13% in MAC versus 8% in SMAC) were comparable between both groups. However, while full myeloablative conditioning might be related to a higher report of events of febrile neutropenia in the EJ population, this does not seem to have an impact on the report of events related to SOC ‘infections and infestations’ or to SOC ‘nervous system disorder. In fact, the higher number of LI patients reporting an infective event in the short and long-term phase compared to EJ (88% LI vs 69% EJ and 88% vs 13% respectively) might be related to the younger age of LI vs EJ and/or a more immature immune system predisposing LI patients to a higher level of infections than older EJ patients.

On the other hand, neurological adverse events associated with MLD such as motor dysfunction, muscle spasticity, aphasia, ataxia, dysarthria, cognitive disorder and seizure were more common in patients who were symptomatic at the time of treatment than in subjects who were pre-symptomatic. In particular, only 6% of subjects in the LI cohort vs 57% of subjects in the EJ cohort were symptomatic at time of gene therapy, and nervous system disorders were observed in 69% of EJ subjects during follow-up post gene therapy compared to 38% of LI during the same period. Given that MAC was the prevalent conditioning regimen used in EJ patients, at present it cannot be ruled out that busulfan as myeloablative condition regimen may contribute to the neurological disorders of these patients. However, the SmPC includes specific text related to the prevention and management of infective and neurological adverse events related to the use of busulfan, in particular about the prophylactic measures to be taken before administering busulfan.

Adverse events by symptomatic status. The integrated safety set population was broadly divided into subjects who were pre-symptomatic or symptomatic at the time of treatment. As expected, subjects who were symptomatic experienced more adverse events due to MLD. There was no different pattern for the non-MLD associated adverse events between the two subgroups.

Adverse events by conditioning regimen

Acknowledging the small numbers of the subgroups, differences are noted between the safety profiles of SMAC versus MAC, with more toxicity observed in the MAC regimen as to be expected. For example, patients treated with MAC experienced more grade 3 AEs in the treatment phase and the total number of events in the three months post-GT phase was also higher. It also seems that the incidences are higher in the MAC vs SMAC regimen for febrile neutropenia and stomatitis and duration of neutropenia was longer in the MAC group.

Adverse events with cryopreserved formulation.

The amount of safety data available for the cryopreserved formulation is too limited to draw any conclusions. However, there is no apparent difference in safety between the fresh formulation and cryopreserved formulation. Also, the provided data in the Quality dossier support comparability between the fresh and cryopreserved DP formulations. At the time of MAA submission, only preliminary safety data from the first 4 subjects treated with Libmeldy-c was available and it was noted that more prolonged severe neutropenia was observed after Libmeldy-c. The mean number of days with an ANC of 0 was 20.7 days (range 15-29 days), much higher than the 4.6 days (range 1.81 to 11.50 days) observed with the fresh product. Based on data from 5 additional subjects treated in Study 205756, no clinically relevant differences were observed in the duration of neutropenia (ANC<500/ul) nor absolute aplasia (ANC=0) between both OTL-200 formulations. The median of days in neutropenia reported was 28 days (range 13 to 39 days) in subjects who received the fresh formulation (N=29) compared with 32 days (range 14 to 40 days) in subjects who received the cryopreserved formulation (N=9). The geometric mean number of days with absolute aplasia (ANC=0) was 4.6 in subjects treated with Libmeldy-f vs. 9.8 days in subjects treated with Libmeldy-c, whereas the range of days in absolute aplasia was comparable for both formulations (Libmeldy-f: range 0 to 28 days; Libmeldy-c: range: 3 to 29 days). No additional subjects treated with the cryopreserved formulation experienced prolonged neutropenia requiring G-CSF treatment. No trend of higher rate of infections was observed in subjects treated with the cryopreserved formulation when compared with the Integrated Data set of subjects treated with Libmeldy-f during the first 3 months post-treatment.

For commercialisation, Libmeldy will be manufactured as a cryopreserved formulation. Additional data indicate that the cryopreserved formulation of Libmeldy shows comparable haematological recovery with the fresh formulation of Libmeldy.

Immunological events

Anti ARSA Antibodies (AAA) have been detected in 4 subjects, all with late infantile MLD, and all from the expanded access programme. For one subject this event resolved spontaneously, for the other 3 cases the events resolved following a course of rituximab treatment. Following the new data-cut, there were two new AAA events: one re-emergence in one subject (Patient 28) and one new case in another subject (LI) from the cryopreserved study. Graphical presentations were provided by the applicant that plots the AAA titres over time against ARSA activity in PBMCs, rituximab treatment, GMFM score/GMFC-MLD level and concurrent adverse events. There were two cases (Patient 25 and 28) where AAAs were observed followed by reporting of gait disturbance. A temporal relationship was acknowledged however no firm conclusions can be drawn considering the very low titres of AAAs in these subjects, the lack of impact on pharmacodynamic effects and

the persistence of motor dysfunction well after AAA turned negative in one subject (Patient 25) or the limited duration of the gait disturbance reported for the other patient (Patient 28). Overall, there appears to be no correlation between the course of the antibody titres, the ARSA activity and clinical outcomes. Moreover, antibody titres were generally low. Further information was provided that indicated that the subject from the cryopreserved study has been AAA negative from the Day 90 scheduled test. At the year 3 visit, Patient 28 still had a positive AAA test but the titre was low (1:400) and no events were reported that coincided with the occurrence that are suggestive of disease progression/lack of efficacy. Additional graphs were provided that plotted ARSA activity in CSF against AAA titres. Based upon the provided plots it appears that the occurrence of AAAs does not impact CSF ARSA activity. Rituximab treatment was initiated in 2 subjects following the detection of AAAs. In the other 2 subjects rituximab treatment was started due to concurrent presence of other auto antibodies. Based upon the data so far, there appears to be no suggestion that the presence of AAAs would impact Libmeldy's efficacy. However, as this only concerns a small sample, no definitive conclusions can be drawn. Recommendations have been formulated on how to monitor AAAs in the SmPC and what to do in case of an AAA positive test. Furthermore, AAAs will continue to be monitored in the proposed PAES. This is currently considered sufficient to address the occurrence of AAAs.

From the perspective of autoimmunity, there are no published cases or reports of MLD causing an autoimmune susceptibility or evidence that autoimmunity would predispose to anti-ARSA antibodies.

Premature study withdrawal due to adverse events

Three subjects (10%) prematurely withdrew from the study.

2.8.2. Conclusions on clinical safety

The safety assessment is based on a limited number of patients (n=35) with a relatively limited duration of follow up in the integrated dataset (median duration 4.51 years (range: 0.64 to 8.85 years) for patients treated with fresh formulation) given the irreversibility of treatment, but the safety of Libmeldy so far is as expected for a haematopoietic stem cell transplantation therapy preceded by myeloablative conditioning regimen. Most of the adverse events observed appear related to the busulfan conditioning regimen. The adverse event attributed to Libmeldy specifically was Anti-ARSA Antibody test positive, and this occurred infrequently and did not appear to impact efficacy thus far.

The CAT considered the following measures necessary to ensure the follow-up of adverse drug reactions:
In order to further characterise the long-term efficacy and safety of Libmeldy in children with late infantile or early juvenile forms of MLD, the MAH will conduct and submit the results of a prospective study based on data from a registry, according to an agreed protocol

The CHMP endorse the CAT conclusion on clinical safety as described above.

2.9. Risk Management Plan

Safety concerns

Important identified risks	Delayed platelet engraftment
Important potential risks	Malignancy due to insertional oncogenesis Anti-ARSA antibodies Engraftment failure Off label use in other MLD subgroups
Missing information	Long-term safety and efficacy data

Pharmacovigilance plan

Study status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
LongTERM-MLD study: Long-term, Efficacy and Safety follow-up of MLD patients treated with *ex vivo* Gene Therapy Using Autologous Hematopoietic Stem Cells Transduced with ARSA Lentiviral Vector (Libmeldy) Planned	To continue to monitor long-term safety and efficacy outcomes data from patients treated with Libmeldy for up to 15 years post treatment	• Delayed platelet engraftment • Malignancy due to insertional oncogenesis • Anti-ARSA antibodies • Engraftment failure • Off label use in other MLD subgroups • Long-term safety and efficacy data	Submission of the full protocol for the LongTERM-MLD study Information on the progress in the identification of a suitable registry FPFV: Interim reports: Final study report:	Within 3 months of the European Commission MA decision With every PSUR 2021 Sep-2023 Dec-2026 Mar-2029 Mar-2034 Mar-2039 31-Mar-2041
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
Study 201222: A Phase I/II clinical trial	To evaluate the safety and efficacy of the fresh	• Delayed platelet engraftment	First patient first visit (FPFV):	09-Apr-2010

Study status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
of hematopoietic stem cell gene therapy for the treatment of Metachromatic Leukodystrophy Ongoing	formulation of OTL-200 in 20 early-onset MLD patients followed up for 8 years after treatment with OTL-200	• Malignancy due to insertional oncogenesis • Anti-ARSA antibodies • Engraftment failure • Off label use in other MLD subgroups • Long-term safety and efficacy data	Interim reports: No.1: No. 2: No. 2.1: No. 2.2: Final study report:	 06-Dec-2017 19-Feb-2019 28-Mar-2019 Sep-2019 31- Mar-2024
Study 205756: A Phase II, single arm, open label, clinical study of cryopreserved autologous $CD34^+$ cells transduced with lentiviral vector containing human ARSA cDNA OTL-200, for the treatment of early onset Metachromatic Leukodystrophy (MLD) Ongoing	To evaluate the safety and efficacy of the cryopreserved formulation of OTL-200 (OTL-200-c) in up to 10 pre-symptomatic, early-onset MLD patients followed up for 8 years after treatment with OTL-200-c	• Delayed platelet engraftment • Malignancy due to insertional oncogenesis • Anti-ARSA antibodies • Engraftment failure • Off label use in other MLD subgroups • Long-term safety and efficacy data	FPFV: Interim reports: Final study report:	25-Jan-2018 14-Mar-2019 31 December 2029
Study OTL-200-07: An open label, non-randomised trial to evaluate the safety and efficacy of a single infusion of OTL-200 in patients with Late Juvenile (LJ) Metachromatic Leukodystrophy (MLD) Planned	To evaluate the safety and efficacy of a single infusion of OTL-200 in patients with Late Juvenile (LJ) Metachromatic Leukodystrophy (MLD)	• Delayed platelet engraftment • Malignancy due to insertional oncogenesis • Anti-ARSA antibodies • Engraftment failure • Off label use in other MLD subgroups • Long-term safety and efficacy data	FPFV: Interim report: Final study report:	Q3 2020 (anticipated) 2028 30 June 2032
CUP 206258: Compassionate use programme for hematopoietic stem cell gene therapy OTL-200 in pre-symptomatic early onset Metachromatic Leukodystrophy patients Ongoing	To provide an alternative treatment option to MLD patients with high unmet need, in advance of OTL-200 being commercially available	• Delayed platelet engraftment • Malignancy due to insertional oncogenesis • Anti-ARSA antibodies • Engraftment failure • Off label use in other MLD subgroups • Long-term safety and efficacy data	FPFV: Interim report: Final study report:	16-Jan-2017 05-Dec-2018 (data cut-off) 31 December 2026
Single-patient CUP 207394 (MLD-C02): Gene therapy protocol	To provide a mechanism to supply OTL-200 on a compassionate use basis to a patient (MLD-	• Delayed platelet engraftment • Malignancy due to	FPFV:	23-Apr-2013

Study status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
using autologous haematopoietic stem cells for MLD-C02, a patient with metachromatic leukodystrophy (MLD) Ongoing	C02) with early symptomatic EJ MLD	insertional oncogenesis • Anti-ARSA antibodies • Engraftment failure • Off label use in other MLD subgroups • Long-term safety and efficacy data	Interim report: Final report:	05-Jan-2018 (data cut-off) 30 September 2022
HE 205029: Hematopoietic stem cell gene therapy for pre-symptomatic Late Infantile Metachromatic Leukodystrophy Ongoing	To provide an alternative treatment option to MLD patients with high unmet need, in advance of OTL-200 being commercially available	• Delayed platelet engraftment • Malignancy due to insertional oncogenesis • Anti-ARSA antibodies • Engraftment failure • Off label use in other MLD subgroups • Long-term safety and efficacy data	FPFV: Interim report: Final study report:	29-Dec-2015 05-Dec-2018 (data cut-off) 31 December 2026

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Delayed platelet engraftment (Important identified risk)	Routine risk minimisation communication • *Information that there have been cases of delayed platelet engraftment in clinical studies in SmPC section 4.4* • *Information that there have been cases of delayed platelet engraftment in clinical studies in PL section 2* Routine risk minimisation activities recommending specific clinical measures to address the risk: • *Warning that Libmeldy may cause delayed platelet engraftment in SmPC section 4.4* Additional risk minimisation measures • *Educational materials for healthcare professionals* • *Educational materials for patients* Other routine risk minimisation measures beyond the Product Information: • *Legal status: Medicinal product subject to restricted medical prescription*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *Review of aggregate safety data* Additional pharmacovigilance activities: • *Study 201222* • *Study 205756* • *Study OTL-200-07* • *CUP 206258* • *Single-patient CUP 207394 HE 205029* • *LongTERM-MLD study*
Malignancy due to insertional oncogenesis	Routine risk minimisation measures:	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:

Safety concern	Risk minimisation measures	Pharmacovigilance activities
(Important potential risk)	• *Information that there have been no cases of leukaemia or lymphoma in SmPC section 4.4* • *Information that no patients have developed leukaemia or lymphoma in PL section 2* • *Information that no abnormal or malignant growth of transplanted cells or hematopoietic tumours were found in a study in mice in SmPC section 5.3* • *Warning that Libmeldy may theoretically cause leukaemia or lymphoma with instructions on blood sample collection if malignancy occurs in SmPC section 4.4* • *Warning that the patient will be asked to enrol in follow up study for up to 15 years and will be monitored for any signs of blood cancer because of the theoretical cancer risk in PL section 2* • *Restricted medical prescription* Additional risk minimisation measures: • *Educational materials for healthcare professionals* • *Patient and parent/carer information pack*	• *None* Additional pharmacovigilance activities: • *Study 201222* • *Study 205756* • *Study OTL-200-07* • *CUP 206258* • *Single-patient CUP 207394* • *HE 205029* • *LongTERM-MLD study*
Anti-ARSA antibodies (Important potential risk)	Routine risk minimisation measures: • *Information that there have been cases of AAAs reported during clinical development in SmPC section 4.4* • *Warning that monitoring for the presence of AAAs is recommended prior to treatment and regularly during post-treatment follow-up in SmPC section 4.4.* • *Guidance on short treatment with rituximab in SmPC section 4.4.* • *Restricted medical prescription* Additional risk minimisation measures: • *Educational materials for healthcare professionals* • *Patient and parent/carer information pack*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Study 201222* • *Study 205756* • *Study OTL-200-07* • *CUP 206258* • *Single-patient CUP 207394* • *HE 205029* • *LongTERM-MLD study*
Engraftment failure (Important potential risk)	Routine risk minimisation measures: • *Information that no patients failed to engraft bone marrow in SmPC sections 4.4 and 5.1*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None*

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	• *Information that following successful and stable engraftment the effects of Libmeldy are expected to be life-long in SmPC section 5.1* • *Information that the doctor will collect two samples of bone marrow or blood including a stem cell backup sample in case Libmeldy does not work in PL section 3* • *Instructions to obtain a CD34⁺ stem cell back-up for use as rescue treatment in SmPC section 4.2* • *Guidance that myeloablative conditioning is required before infusion of Libmeldy to promote engraftment in SmPC section 4.2* • *Warning that in case of cytopenia symptoms, red blood cells and platelet counts should be monitored until engraftment of these cells and recovery are achieved in SmPC section 4.4* • *Guidance to infuse the non-transduced back-up cells if cytopenia persists beyond six to seven weeks in SmPC section 4.4* • *Guidance that in case of engraftment failure, the non-transduced back-up cells should be infused in SmPC section 4.4* • *Guidance that if the modified stem cells do not take hold (engraft) in the patient's body, the doctor may give an infusion of the backup original stem cells in PL section 2* • *Restricted medical prescription* Additional risk minimisation measures: • *Educational materials for healthcare professionals* • *Patient and parent/carer information pack*	Additional pharmacovigilance activities: • *Study 201222* • *Study 205756* • *Study OTL-200-07* • *CUP 206258* • *Single-patient CUP 207394* • *HE 205029* • *LongTERM-MLD study*
Off label use in other MLD subgroups (Important potential risk)	Routine risk minimisation measures: • *Therapeutic indication in SmPC section 4.1 and PL section 1* • *Warning that eligibility to treatment should be assessed by the treating physician in SmPC section 4.4* • *Restricted medical prescription* Additional risk minimisation measures:	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Study 201222* • *Study 205756*

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	• *Educational materials for healthcare professionals* • *Patient and parent/carer information pack* • *Controlled distribution*	• *Study OTL-200-07* • *CUP 206258* • *Single-patient CUP 207394* • *HE 205029* • *LongTERM-MLD study*
Long-term safety and efficacy data (Missing information)	Routine risk minimisation measures: • *Information on the duration of patient follow-up in the clinical studies in SmPC section 5.1* • *Guidance that patients will be asked to enrol in a follow-up study for up to 15 years in SmPC section 4.2 and PL section 2* • *Restricted medical prescription* Additional risk minimisation measures: • *Educational materials for healthcare professionals* • *Patient and parent/carer information pack* • *Controlled distribution*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Study 201222* • *Study 205756* • *Study OTL-200-07* • *CUP 206258* • *Single-patient CUP 207394* • *HE 205029* • *LongTERM-MLD study*

Conclusion

The CHMP, CAT and PRAC considered that the risk management plan version 1.0 is acceptable.

2.10. Pharmacovigilance

Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.11. New Active Substance

The applicant declared that autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells transduced *ex vivo* using a lentiviral vector encoding the human arylsulfatase A gene has

not been previously authorised in a medicinal product in the European Union.

The CAT/CHMP, based on the available data, considers autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells transduced *ex vivo* using a lentiviral vector encoding the human arylsulfatase A gene to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.12. Product information

2.12.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.12.2. Labelling exemptions

A request to omit certain particulars from the labelling as per Art.63.3 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

The applicant requested the use of minimum particulars on small immediate packaging units for infusion bag label. The QRD requested in a first instance to explore if the label on the infusion bag could have double-sided printing which would allow inclusion of the full particulars. If this option is not considered a viable solution, the Group would accept the use of minimum particulars on small immediate packaging units, as the full particulars would be included in the overwrapping and outer labels. Further to additional clarifications from the applicant explaining the challenges of double-sided printing, the Group accepted to have minimum particulars on small immediate packaging units for infusion bag label.

The particulars to be omitted as per the QRD Group decision described above will however be included in the Annexes published with the EPAR on EMA website and translated in all languages but will appear in grey-shaded to show that they will not be included on the printed materials.

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

The applicant requested English-only on immediate, intermediate, outer packaging and lot information sheet. The Group noted that out of the estimated patients to be treated till 2026, a high proportion would be German speaking, therefore dual DE/EN would be preferable to EN only. The Group thereby requested that simplifications to the labelling be considered (e.g. statement on children may be removed; greying out of pharmaceutical form) to explore whether DE/EN labelling could be feasible. Further to additional clarifications from the applicant the Group accepted to have EN only labelling.

The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

2.12.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells transduced *ex vivo* using a lentiviral vector encoding the human arylsulfatase A gene is included in the additional monitoring list as it contains a new active substance which, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Metachromatic Leukodystrophy (MLD) is a rare autosomal recessive inherited lysosomal storage disorder caused by mutations in the ARSA gene that results in deficiency of its corresponding enzyme.

ARSA deficiency results in accumulation of the undegraded substrate, cerebroside 3 sulfate, in lysosomes of oligodendrocytes, microglia, certain neurons of the CNS, Schwann cells and macrophages of the PNS, and other non-neural tissues (e.g., gallbladder, liver, pancreas, and kidneys). Accumulation in the nervous system, in turn, leads to microglial damage, progressive demyelination, neurodegeneration, and subsequent loss of motor and cognitive functions and early death, especially in patients with early disease onset (Bergner, 2019, Gieselmann, 2010, van Rappard, 2015).

There is no universally accepted classification system for MLD phenotypes and at least 3 clinical forms of the disease are commonly described (late infantile [LI], juvenile, and adult MLD) (Von Figura, 2001, Kolodny, 1995). Of note, the juvenile forms are further stratified into early (EJ) and late juvenile (LJ). It is reported that the underlying disease pathophysiology described above is common for all phenotypic forms of MLD (Biffi, 2008a).

Regardless of the clinical classification, the clinical course of the disease can be broadly divided into a pre-symptomatic stage with normal motor and cognitive development, followed by onset of first symptoms and a period of developmental plateau which is short in early onset forms and longer and more variable in late onset forms. In the absence of treatment able to modify the disease pathophysiology, the disease inevitably ends in decerebrated state and eventually death, although its course and duration are highly variable, particularly in late-onset MLD variants (Biffi, 2008b, van Rappard, 2015, Elgun, 2019).

In LI symptoms manifestation starts before 30 months of age. LI patients have the most aggressive form of the disease with a highly predictable and severe disease course, characterised by progressive decline in motor and cognitive function and an early death (Gieselmann, 2010, van Rappard, 2015).

The phenotypic variability is particularly evident in the juvenile and adult variants and is observed in terms of clinical presentation, age at disease onset and dynamics in the rate of disease progression (Biffi, 2008a, Biffi, 2008b, Lugowska, 2005, Polten, 1991). Patients who are affected by the early juvenile (EJ) variant typically

carry one null allele and one residual allele (0/R genotype), have symptom onset between the ages of 30 months and 6 years of age (i.e., have not celebrated their 7th birthday), and tend to have slower and more variable initial disease progression compared to LI MLD (Groeschel, 2016). LJ MLD (age of disease onset ≥7 years and <17 years) and adult MLD patients (age of disease onset ≥17), on the other hand typically carry 2 residual alleles (R/R genotype) and predominantly develop cognitive and behavioral symptoms that can precede the deterioration of gait and motor function and have more prolonged, less rapid disease progression compared to early-onset (LI and EJ) variants (Biffi, 2008a, Biffi, 2008b, Gieselmann, 2010).

The applicant originally proposed a broad indication which included all paediatric MLD disease variants, i.e. irrespective of symptomatic status. However, based on the analysis of the data, it was concluded that efficacy is the greatest in treated pre-symptomatic patients and for early symptomatic EJ MLD patients, prognostic values were identified. These are included in the indication and section 5.1 of the SmPC.

The applicant also removed the LJ MLD disease variant from the indication as full extrapolation was not considered appropriate, due to differences in disease progression, the B/R should be weight differently and, as the efficacy is impacted by the symptomatic status, the prognostic values for these patients are currently unclear as these subjects are currently identified when already symptomatic.

The applicant amended the indication as requested to:

is indicated for the treatment of metachromatic leukodystrophy (MLD) characterized by biallelic mutations in the arysulfatase A (ARSA) gene leading to a reduction of the ARSA enzymatic activity:

- in children with late infantile or early juvenile forms, without clinical manifestations of the disease,

- in children with the early juvenile form, with early clinical manifestations of the disease, who still have the ability to walk independently and before the onset of cognitive decline

(see section 5.1).

This indication will ensure that treatment success is maximized.

3.1.2. Available therapies and unmet medical need

There is currently no curative treatment for MLD. Available treatments only address the symptoms of the disease and none of them have proven a consistent effect on the fatal outcome. Allogeneic haematopoietic stem cell transplantation (HSCT) has been used for the treatment of MLD but results have been inconsistent.

The unmet medical need for patients with MLD has been acknowledged in the applicant's request for accelerated assessment.

In support of this indication, results have been submitted from 33 subjects who were treated in 5 submitted studies by the applicant: 1 registrational study (201222), 3 Expanded access programme studies (207394, 205029 and 206225) and 1 quality comparative study (205756).

3.1.3. Main clinical studies

Study 201222 was a non-randomised, open-label, prospective, comparative (historical control), single centre study in 20 subjects with MLD. Nine subjects were classified as having late infantile (LI) MLD (8 pre-symptomatic) and 11 subjects were classified as early juvenile (EJ) MLD (4 pre-symptomatic).

LI MLD (n=9) was defined as patients with an age at onset in the older sibling ≤30 months, and/or 2 null (0) mutant ARSA alleles, and/or peripheral neuropathy at electroneurographic study. EJ MLD (n=11) was defined as age of onset in the subject or effected sibling between 30 months and 7th birthday, and/or 1 null (0) and 1 residual (R) mutant ARSA alleles, and/or peripheral neuropathy at electroneurographic. These correspond with the general distinction for the MLD variants. All LI MLD and some EJ MLD subjects were identified after an older sibling developed symptoms and was diagnosed with MLD, prompting family testing.

The study consisted of 4 phases, i.e. Screening phase, Baseline phase, Treatment phase and Follow Up phase of 8 years.

The treatment phase included a cell harvest phase for investigational DP manufacture on Day -4 and busulfan conditioning phase (Day -4 to Day -1) and an administration phase where the gene therapy was applied (Day 0). During the study, several changes were introduced by the applicant involving different parts of the treatment phase, these include changes to the drug product manufacturing, addition of peripheral blood (PB) as possible source for CD34+ instead of bone marrow (BM) (for 1 subject PB was used) and a switch from a SMAC busulfan conditioning regimen to a MAC regimen during the study. With the *SMAC* regimen 14 doses of weight-based busulfan were administered every 6 hours from Day -4 to Day -1. A target dose AUC of 4800 µg*h/L (range 4200 to 5600 µg*h/L) was used, corresponding to an expected total cumulative AUC of 67,200 µg*h/L (range 58,800 to 78,400 mg*h/L). For the *MAC* regimen, a total of 4 doses were administered every 20-24 hours from Day -4 to Day -1 with target total cumulative AUC of 85,000 µg*h/L (range 76,500 to 93,500 µg*h/L).

Primary outcomes were total gross motor function measure (GMFM) score and the ARSA Activity in total PBMCs, both at 2 years post treatment. Results were compared to a natural history cohort (NHx cohort), matched by age, MLD variant and symptomatic status.

Secondary endpoints included ARSA activity in BM and CSF, nerve conduction velocity (NCV), Brain MRI score, gross motor function classification for MLD (GMFC-MLD), neuropsychological tests, neurological evaluations, survival, engraftment (lentiviral vector transduced cells, vector copy number/cell). The study included comparison to a natural history cohort (NHx represented by 31 untreated early-onset MLD patients (19 LI and 12 EJ, all symptomatic) and where available untreated siblings (a proportion of subjects were siblings of the patients treated with Libmeldy).

The effect of treatment was analysed for the LI MLD and EJ MLD population separately, as the rate of disease progression is different for these variants. In addition, the symptomatic status may also affect the treatment effect therefore analyses of treatment effect by symptomatic status (‘pre-symptomatic’ or ‘symptomatic’) has also been included.

For the EJ MLD population, the applicant defined early-symptomatic as part of an inclusion criterium, therefore not all EJ MLD patients identified were treated. Early symptomatic was initially defined as within 6 months after the first reported symptom, and later as subjects with an intelligence quotient ≥70 and the ability to walk independently for ≥10 steps. In the discussion of efficacy results, early symptomatic is referred to as patients meeting the eligibility criteria for treatment, i.e. IQ ≥ 85 and GMFC-MLD ≤1.

3.2. Favourable effects

Co-Primary endpoint

ARSA Activity

The mean ARSA activity increased in all treated subjects in the total PBMCs, BM and CSF to levels above (for PBMCs and BM) or within the range (for CSF) observed in healthy subjects within 3 months after treatment. At year 2 post treatment, the mean ARSA activity in the CSF was 0.85 nmol/mg/hr (95%CI 0.58; 1.25) for the *LI MLD* subjects (n=9) and 0.64 nmol/mg/hr (95%CI 0.37; 1.13) for the *EJ MLD* subjects (n=11). In the individual profiles fluctuations and drops of ARSA activity levels in the CSF can be noted.

Gross Motor Function Measurement

For the *Late Infantile MLD* 2 years post-treatment, mean total GMFM score in the Libmeldy treated LI MLD subjects was 72.5% compared to 7.4% for the LI MLD NHx subjects (Difference 65.1 %, 95%CI 41.6%; 88.6%), p<0.001).

For the overall *Early Juvenile (EJ)* MLD subjects (n= 11) the mean total GMFM score at 2 years was 76.5% for the Libmeldy treatment group compared to 36.6 % for the *EJ MLD* NHx cohort (Difference 39.8 % 95%CI 9.6%; 80.1%, p=0.026).

The subgroup of *pre-symptomatic EJ MLD (n=4)* had an adjusted mean GMFM total score of 96.7%, while for the NHx a mean GMFM total score of 44.3% was reported. The difference between the Libmeldy treated and corresponding NHx subjects was 52.4% (95%CI 25.1%; 79.6%, p=0.008) at Year 2. In the subgroup of *symptomatic EJ MLD (n=8) a*t Year 2 and Year 3 there was a difference in treatment effect of 28.7% (60.7% vs 31.9% in the untreated NHs control; p=0.350) and 43.9% (59.8% vs. 15.9%; p=0.054), respectively. At Year 4 and Year 5 this difference was confirmed (42.9% for both: 53.6% vs. 10.7% [p=0.054] and 50.3% vs. 7.4% [p=0-107], respectively).

Secondary outcomes

Engraftment of Lentiviral vector in BM

Engraftment of bone marrow derived colonies harbouring the lentiviral genome was successful in all treated subjects. Levels of cells containing the Lentiviral vector in the BM increased within 28 days to 59.1% and was 54.8% (CI 95% 44.1; 68.2%, [n=23]) at Year 1 post treatment. At Year 5, that proportion was 45.0% (CI95% 24.1%; 84.2% [n=6]). The VCN in the PBMCs increased to approximately 1 VCN/cell for the LI MLD and approximately 0.25 VCN/cell for the EJ MLD.

VCN values in total PBMCs had a geometric mean of 0.19 copies/cell (range 0.03 to 0.68; n=29) at Day 28 post gene therapy. In both the *LI MLD* and *EJ MLD* subgroups, the VCN in total PBMCs increased over time until 3 months and remained relatively stable throughout the course of follow-up.

Brain MRI

For the *LI MLD* subject the MRI outcome was consistent with the GMFM scores, i.e. the MRI score mean differences between Libmeldy treated *LI* subjects and NHx subject of -11.8 (p<0.001). Individual plots show stabilisation over time.

For the *EJ MLD* the difference between the Libmeldy treatment group and NHx was -4.1 points, p=0.12, on brain MRI. For the *pre-symptomatic EJ MLD* subject the differences in the adjusted LS mean MRI total scores between the Libmeldy treated pre-symptomatic EJ subjects and NHx subjects was 10.7 (95%CI 7.0, 14.4; p<0.001). For the *symptomatic EJ MLD* patients the difference in LS mean MRI total score between the Libmeldy treated symptomatic subjects and NHX was 5.8 (95% CI -4.0, 15.5; P=0.21). Individual plots in symptomatic and pre-symtomatic EJ subjects show stabilisation over time.

Cognitive performance

The cognitive performance was within range reported for healthy subjects for the majority of the *LI MLD* subjects.

For the *EJ MLD*, all 4 subjects who were treated prior to the onset of symptoms had neuropsychological composite scores that were largely within or above the normal range (score of 100 +/- SD of 15) at the time of the data cut-off, with the exception of the Processing Speed Index.

Throughout the extended follow-up period, 6/8 Early Symptomatic EJ subjects remained stable and above the threshold for sever cognitive impairment (IQ≥55) with difference from the NHx controls at Year 2 (IQ 88.9 vs. 31.9 in NHx controls; treatment difference 61.1, p=0.029), Year 3 (89.4 vs. 17.6; treatment difference 71.8, p=0.013), Year 4 (81.9 vs. 15.2; treatment difference 66.8, p=0.026), but not at Year 5 (48.2 vs. 9.8).

Survival

A borderline statistically significant difference was observed for overall survival of the *LI MLD* subjects as compared to the NHx cohort (0/9 vs 12/19, unstratified Log-rank p-value 0.062) while no statistically significant differences were found for the *EJ MLD* subjects (estimated HR 1.85; log rank p-value= 0.537). In the *EJ MLD* subgroup 2 deaths occurred, due to disease progression. Both concern patients that were early symptomatic at start of treatment and who would not meet the current proposed criteria for treatment with Libmeldy.

The expanded access programmes included *pre-symptomatic LI MLD* and *EJ MLD* patients only. The data from these confirm the effect on motor- and cognitive function if the ARSA levels in the CSF are maintained above a threshold. Here also a fluctuation in CSF ARSA activity levels is observed.

3.3. Uncertainties and limitations about favourable effects

Historically, the product has been manufactured as a fresh (i.e. non-frozen) formulation using bone marrow (BM) as starting material. In the MAA, the applicant also applies for the use of mobilised peripheral blood (mPB). This is sufficiently supported by comparability data. Some immunophenotypic differences in cell composition are observed and VCN and ARSA activity in batches manufactured from mPB was on average lower in mPB-derived batches. VCN and ARSA activity was, however, within the range observed for BM-derived batches. From the clinical data there are no indications that the immunophenotypic differences result in differences in clinical outcome. It is noted, that analytical results and adverse trends will be further monitored as part of the CPV programme. In addition, follow-up data will be gathered for the patients treated with mPB derived batches.

During development, several changes were made to the manufacturing procedure of the vector and the Drug Substance and the Drug Product formulation was changed to a cryopreserved product. Sufficient data are provided to support comparability. Although considerable batch-to-batch variability in e.g. VCN is observed, in general the differences cannot be linked to differences in DS/DP or vector manufacturing procedures. There are no indications that the changes in manufacturing procedure have negatively impacted product quality or clinical efficacy. The slightly lower cell viability in the cryopreserved DP is expected and gives no reason for concern.

A more in-depth evaluation of VCN in DP batches during development was performed as there was concern that higher VCN in more recent batches may be associated with lower ARSA activity in CSF and higher MRI scores after 1 year of treatment. The results showed that there has been no drift towards higher VCN with the commercial process. A comparison of the clinical data showed no negative impact of high DP VCN on

engraftment, ARSA activity in CSF and PBMC, MRI scores, or clinical efficacy (motor function). However, the data also show that that levels of cells with VCN> 10 were considerably lower in the patient samples 1 year after treatment compared to DP, suggesting preferential engraftment of subpopulations with low VCN or loss of cells with higher VCN. The potential disappearance of cells with high VCN in the patients could give rise to a concern with respect to sustained efficacy. In addition, the high percentage of cells with VCN > 10 (up to 44% in DP batches) could be a reason to be cautious with respect to safety. It is therefore considered important to continue analyzing the circulating cells (and their VCN) in the patients. The applicant has committed to this in a post-authorisation study, however, only a synopsis was submitted. If sufficient data are available, the DP specifications should be re-evaluated taking into account the results of the clinical monitoring (e.g. VCN, ARSA activity, clinical efficacy). In addition to the changes in the manufacturing process mentioned above, the applicant also changed the busulfan conditioning regimen. The data in the Libmeldy studies do not show clear differences in terms of engraftment efficiency and ARSA activity between SMAC and MAC and it is not possible to have one of the regimens as preferred choice. The choice should be left to the treating physician based on an individual benefit/risk assessment.

The primary efficacy endpoints, i.e. ARSA activity and GMFM total score, were assessed at a fixed time point at 2 years post treatment. With respect to the GMFM the longitudinal evaluation is considered more appropriate, as this gives a better representation of the overall course of the efficacy in relation to the disease progression. For the majority of the *LI MLD* the effect observed on the GMFM at 2 years is also reflected in the overall longitudinal pattern. In contrast for all symptomatic *EJ MLD* subjects on group level a difference of 28.7% from the NHx is observed 2 years post treatment, while the individual panel plots showed deterioration on GMFM. The deterioration on individual level was slowed or halted in subject who would meet the current treatment criteria. This deterioration was also observed in other outcomes, e.g. cognitive function, survival etc.

However, for symptomatic patients meeting the proposed criteria of IQ≥85 and GMFC-MLD ≤1, the cognitive functions appear to be maintained as these subjects perform equal to their healthy peers. These criteria are based on limit data but are accepted by lack of better. If more follow up data becomes available these criteria may need to be adapted. Further identification of prognostic factors for response, i.e. target population, should be part of the post-authorisation study.

Effects on group level are diluted due to wide inter-individual variability for the different clinical outcomes. Moreover, the individual profiles for different clinical outcomes of the symptomatic subjects indicate disease progression, particularly for motor function. However, as cognitive function can be preserved if treated early, there may be window of opportunity. Therefore, the criteria should be evaluated regularly and adjusted if the new available data suggest this.

In the individual plots for ARSA activity in the CSF a decrease in ARSA activity levels can be noted which cannot be explained by assay variability. It remains unclear if the ARSA activity levels in the CSF are maintained over time as the decrease in ARSA levels observed were in the last part of the follow up. The applicant hypothesized that as the ARSA activity is normalized based on total CSF protein the decrease notes is due to an increase in protein in the CSF over time in MLD subjects. This could not be verified. The applicant has indicated to further evaluate the relationship between CSF protein content and ARSA activity. This would allow evaluation of ARSA CSF activity as predicting factor for treatment success or explanation of failure.

No consistent correlation between the reconstitution of normal ARSA activity and clinical benefit in terms of decrease of gross motor function deterioration could be determined, which questions the ability of the ARSA

activity as co-primary endpoint to predict clinical benefit. Similarly, no statistically significant correlations were found between engraftment parameters and clinical outcome measures (i.e. GMFM at Year 2 and Year 3 timepoints post-treatment).

Statistically significant correlations were found between VCN and enzymatic activity in PBMCs at year 2 and Year 3 post-treatment while no correlation was observed between engraftment parameters and ARSA activity in CSF.

Two years post treatment, the time point analysis for the MRI for LI MLD, EJ MLD and the pre-symptomatic and symptomatic EJ MLD subgroups, corresponds with the group effects seen in GFMF at 2 years post treatment. However, it is noted that the individual longitudinal MRI data do not correlate well with the individual clinical outcome as for some subjects, deterioration is observed in the longitudinal data from GMFM and other secondary outcomes, while the MRI score remained stable from 2 years post treatment to the data cut off.

The quality of life was not directly assessed, however, data in the treated pre-symptomatic subject from both school performance and parent reported outcomes indicate that these subjects perform as healthy peers and go about their daily activities without special assistance.

The duration of follow-up is considered limited for this type of gene therapy and this precludes to draw definite conclusions on the long-term efficacy in terms of persistence of engraftment levels of LVV transduced cells, PD parameters (namely, central and peripheral ARSA activity levels), and of treatment effects on clinical (including neuroimaging and neurophysiology) outcome measures as well as on the long-term safety particularly in terms of insertional mutagenesis and oncogenesis. The applicant will address these further in a post-authorisation study.

The reason for the observed numerical differences in engraftment levels of LV+ $CD34^+$ cells and VCN per cell, between LI and EJ patients and by disease stage, is not understood at present, although it could be hypothesized a role for the type of conditioning regimen in the two MLD variants. In addition, a trend towards a reduction in the proportion of LV+ cells in BM over time was observed in both LI and EJ patients, that could be interpreted as a possible indicator for time-dependent decrease in treatment effect.

Efficacy in pre-symptomatic LI MLD and EJ MLD subjects was already concluded previously. For the symptomatic patients the efficacy was questioned as deterioration was observed in all symptomatic patients on motor function. However, data in early symptomatic EJ-MLD subjects indicate that the rate of motor function decline is decreased and may even be halted. Furthermore, for these early symptomatic EJ-MLD patients the cognitive function is maintained as subjects perform within or above ranges observed for heathy subjects of the same age. This observation is in line with the progression of EJ MLD, which starts with loss of motor function followed by cognitive function. It thus appears that there is a window of opportunity for symptomatic EJ MLD population. The maintenance of cognitive function is considered a clinical benefit of the treatment. The prognostic factors for treatment success in early symptomatic subjects will be further investigated post marketing. Currently, MLD is not included in a postnatal screening programme. Therefore, pre-symptomatic patients are only identified if there is an affected sibling. The majority of new MLD subjects will be symptomatic.

The indication proposed by the applicant is largely agreed and includes pre-symptomatic LI-MLD and EJ-MLD and a description of early symptomatic MLD EJ-MLD. However, for clarity, it has been specified that patients should only have early clinical manifestations of disease.

Importantly, Reference to section 5.1 of the SmPC is made as this section includes a more elaborate description of the MLD subvariants, and the definition of early symptomatic.

The data suggests that in early symptomatic EJ MLD the cognitive function appears to be preserved when treated before the onset of cognitive decline. Thus, treatment may be beneficial to preserve cognitive function, while the effect on deterioration of motor function may be limited to a decline in rate of deterioration. This information has been included in section 5.1 of the SmPC as well as treatment of symptomatic LI appears not effective as deterioration in line with normal disease progression is observed.

To allow more rapid treatment of patients, the applicant initially proposed to use a 2-stage release testing strategy, in which product identity (presence of transgene) and potency would not be confirmed to administration.

After evaluation of the available data, it was concluded that it is not justified to maintain the 2-stage release strategy as this is accompanied by a risk of administering a sub-potent medicinal product which may lead to a suboptimal treatment effect, and that retreatment with a new batch is not possible for the patient. The change to a conventional (1-stage) release strategy will, however, result in a 3-4 week longer time interval between screening and treatment compared to the clinical studies. Even if the risk of deterioration of the patient's clinical status between screening and treatment because of this delay could be low, it is preferable to treat the patients as early as possible. Therefore, the applicant is required to reduce the time from screening to treatment towards the ranges used during clinical development within 1 year following approval. Reduction of the time needed for product testing and batch release should be part of the measures to achieve this.

3.4. Unfavourable effects

In general, unfavourable effects reported in the clinical development programme were attributed by the applicant to 3 categories: related to the busulfan conditioning regimen, related to Libmeldy, and related to MLD.

Grade ≥ 3 or higher and other serious adverse events reported were most frequently attributed to either the busulfan conditioning regimen or the underlying arylsulfatase deficiency. All grade ≥3 infections occurring within the first 3 months resolved and none was reported as SAE.

In the 3-month post gene therapy phase, the most commonly occurring adverse events were attributable to busulfan conditioning and consisted of: febrile neutropenia (79%), stomatitis (41%) and mucosal inflammation (34%).

In the subsequent treatment phases (short and long term) adverse events related to MLD are most often reported: gait disturbance (31%), motor dysfunction (24%), muscle spasticity (31%), ataxia (10%), aphasia (14%), dysarthria (14%) and dysphagia (10%).

The only adverse event reported as related to Libmeldy treatment is the presence of anti-ARSA antibodies (AAAs), which were detected in 4 subjects from the expanded access programme. For one subject the AAAs resolved spontaneously, whereas three subjects required treatment with rituximab. Two additional events were identified in May 2020: one new case in study 205756 and one case of re-emergence.

Based on the type of product replication competent lentivirus (RCL) and insertional oncogenesis are theoretical safety concerns. There were no confirmed positive results for replication competent lentivirus. In addition, there was no sign of clonal dominance or clonal proliferation from insertion site analyses. Furthermore, no malignancies were reported during the long term follow up after treatment with Libmeldy.

Three deaths occurred in the clinical development programme. Two subjects died from disease progression and are regarded as treatment failures. One subject died due to an ischemic cerebral infarction a year after gene therapy. A relationship between the event and gene therapy appears unlikely.

3.5. Uncertainties and limitations about unfavourable effects

The number of patients in the Libmeldy safety set is limited. However, the profile seems consistent with the known safety profile of autologous HSCT and busulfan conditioning. Thus, the small patient population does not present an issue for understanding the safety profile of Libmeldy treatment.

The current median follow-up duration of Libmeldy is approximately 3 years, ranging from 1 to 7 years. This is insufficient to allow for evaluation of the long-term safety of Libmeldy and the busulfan conditioning regimen, in particular for the development of malignancies.

3.6. Effects Table

Table 25 Effects Table for Libmeldy.

Effect	Short Description	Unit	Treatment (Libmeldy)	Control (NHx)	Uncertainties/ Strength of evidence	References
Favourable Effects						
Engraftment	VCN/Cells in PBMC 2 years post GT	mean	LI-MLD 0.6744 EJ-MLD 0.5008	NA NA	SoE: 95% CI: 0.3068;1.482 SoE: 95% CI: 34.06; 65.16 Unc: effect of source material, dose, busulfan regimen unknown	201222
ARSA activity	Mean ARSA activity measured in CSF 2 years post GT	nmol/mg/hr	LI-MLD 0.852 EJ-MLD 0.64	NA NA	SoE: 95%CI: 0.581; 1.251 SoE: 95%CI 0.37; 1.13 Unc:unknown if fluctuation is within assay variability. Unknown if remain stable. Unknown what threshold is associated with efficacy	201222

Effect	Short Description	Unit	Treatment (Libmeldy)	Control (NHx)	Uncertainties/ Strength of evidence	References
GMFM	Mean GMFM measured 2 years post GT	%	LI-MLD 72.5% EJ-MLD 76.5% PS EJ-MLD 96.7% S EJ-MLD 60.7%	LI-MLD 7.4% EJ-MLD 36.3% PS EJ-MLD 44.3% S EJ-MLD 32%	SoE: δ=65.1;p<0.001 SoE: δ= 39.8; p=0.026 SoE: δ=52.4%; p=0.008 SoE: δ=28.7%; 0.35 Unc: time point not representative for the overall course Unc: high inter individual variability, as S EJ includes both early and late symptomatic EJ	201222
MRI	Morphological assessment		LI-MLD 2.1 PS EJ-MLD 5.0 S EJ-MLD 13.5	LI-MLD 13.9 PS EJ-MLD 15.7 S EJ-MLD 19.3	SoE: δ = -11.8; <0.001 SoE: δ =-10.7;p<0.001 SoE: δ =-5.8;p=0.21 Unc: time point not representative for the overall course Unc: does not reflect clinical outcomes	201222
Survival	subjects with event, died.		LI-MLD 0/9 EJ-MLD 2/11	LI 12/19 EJ-MLD 3/12	SoE: log rank P=0.062 SoE: : log rank P=0.537 SoE: Survival also confirmed by quality of life	201222
Unfavourable Effects						
Common AE in the 3-month post GT phase (ISS)	AEs occurring in at least 30% of subjects	% (n)	Febrile neutropenia 79% (23) Stomatitis 41% (12) Mucosal inflammation 34% (10)	NA	Most AEs are consistent with known AEs associated with busulfan conditioning	

Effect	Short Description	Unit	Treatment (Libmeldy)	Control (NHx)	Uncertainties/ Strength of evidence	References
Common AE in the short term follow up phase (ISS)	AEs occurring in at least 20% of subjects	% (n)	Gait disturbance 31% (9) Upper respiratory tract infection 38% (11) Motor dysfunction 24% (7) Ear infection 24% (7) Blood IgE increased 21% (6)	NA	Several AEs identified that are associated with MLD.	
Common AE in the long term follow up phase (ISS)	AEs occurring in at least 10% of subjects	% (n)	Upper respiratory tract infection 31% (5) Vitamin D decreased 25% (4) Pyrexia 19% (3) Muscle spasticity 13% (2) Head injury 13% (2) Osteoporosis 13% (2) Scarlet fever 13% (2)	NA	Limited safety data available (n=16) Duration of follow up (median: 3 years)	
Anti Arsa Antibody test positive	Positve anti-arsa antibody test	% (n)	14% (4)	NA	Relationship to/and impact on Libmeldy treatment unclear	

Abbreviations: AE= adverse event, ARSA= arylsufatase A, δ= difference, EJ= early juvenile, GMFM=gross motor function measure, GT= gene therapy, ISS= integrated safety set, LI= late infantile, MLD= metachromatic leukodystrophy, MRI= magnetic resonance imaging , NA=not applicalble, PBMC= peripheral blood mononuclear cells, PS=pre-symptomatic, S=symptomatic, SoE=strength of evidence, Unc=uncertainties, VCN=vector copy number.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The effect of Libmeldy in the pre-symptomatic LI-MLD and pre-symptomatic EJ-MLD is impressive as performance for both motor and cognitive function remains within the normal range of healthy subjects. It is likely that ARSA activity levels need to be maintained above a certain threshold for efficacy. However, the exact level or range of this threshold could not be determined by the applicant. Importantly, a fluctuation in ARSA activity levels in the CSF is observed with a decrease at the end of the follow up for some subjects. The applicant speculated that this could be due to an increase in total CSF proteins, however this could not be verified. This raises concern on regarding the maintenance of ARSA activity levels in the CSF in time at levels where functional effect can be expected, therefore longer-term follow-up data will be required to establish persistence of a treatment effect over a prolonged period of time.

Importantly, patient identification is an issue as Libmeldy shows an impressive effect in pre-symptomatic LI MLD and EJ MLD subjects, and treatment effect is less when patients have become symptomatic and seems to be limited or even lost when patients already show signs of cognitive impairment and/or significant walking disabilities at the time of treatment. In the absence of a screening programme and no clear classification of the MLD variants (i.e. LI-MLD, EJ-MLD and LJ-MLD), it is not clear how pre-symptomatic MLD patients, with no symptomatic sibling already diagnosed with MLD, may be identified for treatment.

As patients may benefit from rapid treatment, the applicant initially proposed to use a 2-stage release testing strategy, in which product identity (presence of transgene) and potency would not be confirmed to administration. As such a strategy can only be accepted under exceptional circumstances, this was subject of extensive discussion. After evaluation of the available data, the CAT concluded that it is not justified to maintain the 2-stage release strategy, as the 3-4-weeks difference in treatment onset compared to a conventional (1-stage) release strategy is unlikely to impact the benefit-risk of the product, as disease progression is still relatively slow (progression from GMFC-MLD level 1 to level 2 in the EJ-MLD population is 1.1 years (10% quartile-90% quartile: 0.34-3.7)). The change to a conventional (1-stage) release strategy will, however, result in a 3-4 weeks longer time interval between screening and treatment compared to the clinical studies. The MAH was therefore requested, to take measures to reduce the overall time from patient screening to treatment to within the ranges observed during clinical development (median 8.2 weeks; range 6-12.4 weeks). Reduction of the time needed for product testing and release should be part of these measures.

Sufficient data are provided to support the changes to the manufacturing process during development, including the change to a cryopreserved formulation and the use of mPB as starting material in addition to BM. The observed differences could not be linked to differences in manufacturing procedure and/or are not expected to result in differences in clinical outcome. However, considerable batch-to-batch variability is observed in VCN and the relatively high percentage of cells with VCN > 10 in some DP batches could give rise to a concern with respect to safety and sustained efficacy. It is therefore considered important to continue analyzing the circulating cells (and their VCN) in the patients. The applicant has committed to further analyze this data. The applicant confirmed that re-evaluation of the DP specifications as part of the annual product review will also take into account the clinical outcome data.

The number of patients in the Libmeldy safety set is limited, and interpretation is hampered by uncertainties. However, the safety profile of Libmeldy in the immediate phases largely resembles from what is expected of

HSCT procedures with busulfan conditioning, which is reassuring. In the subsequent long term follow up phases, more events are reported that are associated with MLD.

While, the engraftment of transduced HSCP was successful in all subjects, it appears that on a group level the engraftment was more successful in subjects with the LI variant, compared to the EJ variant. The cause for this difference is unknown.

The assessment of the long-term safety of Libmeldy treatment, particularly for the development of malignancies, is hampered by the limited safety follow up (median: 4.51 years (range: 0.64 to 8.85 years). For gene therapies with genome editing properties, the safety and efficacy follow-up should be 15 years. A post-authorisation study has been proposed to address this issue and to increase patient numbers, however no formal assessment can be performed as no draft protocol has been submitted.

3.7.2. Balance of benefits and risks

While an impressive effect is observed in the pre-symptomatic LI-MLD and pre-symptomatic EJ –MLD subjects, efficacy, the magnitude of effect in symptomatic EJ-MLD subjects is less evident and more varied. The data on motor function in all symptomatic EJ MLD subjects indicate a deterioration albeit at a possibly somewhat slower rate of decline than untreated subjects, whereas cognitive function seem to be maintained. This observation is in line with the disease process of EJ MLD, which starts with loss of motor function followed by cognitive function. It is therefore concluded that for the early symptomatic EJ MLD population the window of opportunity to benefit from treatment is not immediately lost upon presentation of symptoms.

Taking these results into consideration, it is concluded that in children with the early juvenile form, with early clinical manifestations of the disease, treatment should only be initiated if the patient has the ability to walk independently (the patient's GMFC-MLD score is ≤ 1), and before the onset of cognitive decline (patient's IQ is ≥ 85.) These requirements are reflected in the indication and the product information and will be regularly evaluated and adjusted, if needed, based on data from ongoing and planned post-authorisation safety and efficacy studies.

Based on the considerable batch-to-batch variability in VCN and the concern with respect to safety and sustained efficacy associated with the relatively high percentage of cells with VCN > 10 it is considered important to continue analysing the circulating cells (and their VCN) in the patients. This will be part of the study (see comments below).

In addition, as patients will benefit from rapid treatment, the applicant committed to reduce the time needed between screening and treatment by, amongst others, the introduction of a rapid potency assay.

The safety of Libmeldy so far is as expected for a haematopoietic stem cell transplantation therapy including myeloablation.
Uncertainties remain regarding the long-term safety and the presence of anti-ARSA antibodies, but these could be potentially overruled by the efficacy from the pre-symptomatic LI and EJ subjects and early symptomatic EJ subjects.

In light of the small study population studied and limited follow-up duration, a post-authorisation efficacy and safety study, according to an agreed protocol will continue to monitor and evaluate the maintenance of efficacy and the long-term safety of Libmeldy for up to 15 years post treatment. It is considered necessary to obtain this in both already treated as well as newly treated patients in order to generate a sufficient database that allows conclusions in this orphan population. This in particular applies to motor-function development, cognitive performance of patients as well as occurrence of secondary malignancies. In addition, a number of

planned and on-going post-authorisation studies will monitor safety concerns of delayed platelet engraftment, malignancy due to insertional oncogenesis, Anti-ARSA antibodies, engraftment failure, off-label use in other MLD subgroups and in general, long-term safety and efficacy data.

3.8. Conclusions

The overall B/R of Libmeldy is positive.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described above.

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the benefit-risk balance of Libmeldy is favourable in the following indication:

Libmeldy is indicated for the treatment of metachromatic leukodystrophy (MLD) characterised by biallelic mutations in the arysulfatase A (ARSA) gene leading to a reduction of the ARSA enzymatic activity:

- in children with late infantile or early juvenile forms, without clinical manifestations of the disease,
- in children with the early juvenile form, with early clinical manifestations of the disease, who still have the ability to walk independently and before the onset of cognitive decline.

The CAT therefore recommends the granting of the marketing authorisation subject to the conditions specified below.

Based on the draft CHMP opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP also considers by consensus that the benefit-risk balance of Libmeldy is favourable in the above indication.

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in

the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regards to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

Prior to launch of Libmeldy in each Member State, the MAH will agree about the content and format of the educational and controlled distribution programme with the National Competent Authority.

The educational and controlled distribution programme is aimed at providing information on the safe use of Libmeldy.

The MAH shall ensure that in each Member State where Libmeldy is marketed, all healthcare professionals and patients/carers who are expected to prescribe, dispense and/or use Libmeldy have access to/are provided with the following educational package:

- Physician educational material

- Patient information pack.

The physician educational material should contain:

- The Summary of Product Characteristics

- The Guide for healthcare professionals

- The Guide for handling and method of administration.

The Guide for healthcare professionals shall contain the following key elements:

- Warning that there is a theoretical possibility that the treatment with Libmeldy may be associated with the risk of insertional mutagenesis, potentially leading to development of malignancy. All patients should receive monitoring for signs and symptoms of oncogenic transformation, leukaemia or lymphoma; and must be advised on the symptoms and signs of leukaemia or lymphoma and to seek immediate medical attention if they develop any of the symptoms.
- Warning about delayed platelet engraftment and guidance on its management
- Warning about emergence of anti-ARSA antibodies and guidance on its management
- Warning about the potential risk of engraftment failure and the need to monitor patients
- Information on LongTERM-MLD study and what it will involve
- Recommendation of the important considerations to discuss with patients and/or carers about Libmeldy:
    - Potential risks of a treatment with Libmeldy
    - Signs of any malignancy such as leukaemia/lymphoma and what action to take
    - Content of the patient and parent/carer guide
    - The need to carry the patient alert card and to show it to every healthcare professional
    - The importance of regular monitoring and long-term follow-up.
- Provision of contact details for reporting all suspected adverse reactions and to include the individual medicinal product lot number which can be found within the patient alert card.

The Guide to handling and method of administration for healthcare professionals shall contain the following key elements:

- Guidance that Libmeldy must be administered in a Qualified Treatment Centre with experience in haematopoietic stem cell transplantation (HSCT)
- Instructions on the precautions to be taken before handling or administering Libmeldy
- Instructions for receiving and storing Libmeldy
- Instructions to check Libmeldy prior to administration
- Instructions for the thawing of Libmeldy
- Provision of contact details for reporting all suspected adverse reactions and to include the individual medicinal product lot number which can be found within the patient alert card.

The patient information pack should contain:

- The Package Leaflet

- The Patient and parent/carer guide

- The Patient alert card.

The patient and parent/carer guide shall contain the following key messages:

- Warning to monitor the patient for symptoms of leukaemia or lymphoma and to contact the specialist doctor immediately in case of any symptoms as there is a small risk that a patient may develop leukaemia or lymphoma. The specialist doctor will check the patient’s blood for any signs of leukaemia or lymphoma during the routine yearly check-ups, which will continue after treatment.

- Guidance about the need for the patient or their parent/carer to carry the patient alert card to inform any treating healthcare professional that the child was treated with Libmeldy.
- Guidance on the importance of regular monitoring and to report any symptoms or concerns to the specialist doctor treating the child.
- Information about the LongTERM-MLD study and the purpose of the study.
- Provision of contact details for reporting any side effects or symptoms of the patient and what a medicine subject to additional monitoring (▼) means.

- The patient alert card shall contain the following key messages:
  - Statement that the patient was treated with Libmeldy, with the medicinal product lot number and treatment date to ensure traceability as per the Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products (EMEA/149995/2008).
  - Contact details of the treating physician.
  - Information on the possibility of false positivity of certain commercial HIV tests because of Libmeldy.
  - Statement that the patient was treated with gene therapy and should not donate blood, organs, tissues, or cells.
  - Details on reporting of adverse reactions and that Libmeldy is subject to additional monitoring▼.
  - Contact details where a healthcare professional can receive further information.

The MAH shall ensure that, in each Member State where Libmeldy is marketed, a system aimed to control its distribution beyond the level of control ensured by routine risk minimisation measures is implemented. The following requirements need to be fulfilled before the product is prescribed, manufactured, dispensed and used:

Libmeldy will only be available through treatment centres qualified by the MAH to ensure traceability of the patient's cells and manufactured drug product between the treating hospital and manufacturing site. The selection of the treatment centres will be conducted in collaboration with national health authorities as appropriate. The healthcare professionals will receive training on the physician educational materials as part of the centre qualification process.

Obligation to conduct post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
In order to further characterise the long-term efficacy and safety of Libmeldy in children with late infantile or early juvenile forms of MLD, the MAH shall conduct and submit the results of a prospective study based on data from a registry, according to an agreed protocol.	Interim reports to be submitted in accordance with the RMP

	Final study report: 31 March 2041
The MAH should take measures to reduce the overall time from patient screening to treatment to within the ranges observed during clinical development (median 8.2 weeks; range 6-12.4 weeks). Reduction of the time needed for product testing and release should be part of these measures.	Progress report: June 2021 Report on implementation of measures: December 2021

The CHMP endorse the CAT conclusion on the obligation to conduct post-authorisation measures as described above.

New Active Substance Status

Based on the CAT review of the available data, the CAT considers that autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells transduced *ex vivo* using a lentiviral vector encoding the human arylsulfatase A gene is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

The CHMP endorse the CAT conclusion on the new active substance status claim.