20 May 2021
EMA/332184/2021
Committee for Advanced Therapies (CAT)
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Skysona

International non-proprietary name: elivaldogene autotemcel

Procedure No. EMEA/H/C/003690/0000

Note

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

List of abbreviations

ABCD1	ATP-Binding Cassette, Subfamily D Member 1
AE	Adverse Event
allo-HSCT	Allogeneic Haematopoietic Stem Cell Transplantation
ALD	Adrenoleukodystrophy
ALDP	Adrenoleukodystrophy Protein
AMN	Adrenomyeloneuropathy
ATP	Adenosine Triphosphate
ALT	Alanine Aminotransferase
APOB	Apolipoprotein B Gene
AST	Aspartate Aminotransferase
ATMP	Advanced Therapy Medicinal Product
AUC	Area Under The Curve
BMCs	Bone Marrow Cells
C22:0	Behenic Acid
C26:0	Hexacosanoic Acid
CAT	Committee for Advanced Therapies
CALD	Cerebral Adrenoleukodystrophy
CD14+ %ALDP+ Cells	Percentage of Cells Expressing ALDP in CD14+ Monocytes
CD14+ VCN	VCN Measured in CD14 + Monocytes
cDNA	Complementary Deoxyribonucleic Acid
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
CIF	Cumulative Incidence Function
CSR	Clinical Study Report
CV	Coefficient of Variation
DNA	Deoxyribonucleic Acid
DP %LVV+	Percentage of Cells Transduced in Drug Product
DP VCN	Drug Product Vector Copy Number
EBMT	European Society for Blood And Marrow Transplantation
EC	Ethics Committee
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
Gag	Group-Specific Antigen
G-CSF	Granulocytecolony-Stimulating Factor
GdE	Gadolinium Enhancement
gDNA	Genomic DNA
GMO	Genetically Modified Organism
GVHD	Graft-Versus-Host Disease
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPC-A	Haematopoietic Progenitor Cells-Apheresis
HSC	Haematopoietic Stem Cell
HSCT	Haematopoietic Stem Cell Transplantation
HTA	Health Technology Assessment

IS	Integration Site
ISA	Integration Site Analysis
ITT	Intent-To-Treat
IVIM	Imortalisation Assay
LAM-PCR	Linear Amplification PCR
LOD	Limit of Detection
LOQ	Limit of Quantitation
LVV	Lentiviral Vector
MAA	Marketing Authorisation Application
MFD	Major Functional Disability
MRI	Magnetic Resonance Imaging
MSD	Matched Sibling Donor
NFS	Neurological Function Scale
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
NMSD	Not A Matched Sibling Donor
NE	Neutrophil Engraftment
nrLAM-PCR	Non-Restricted Linear Amplification PCR
[nr]LAM-PCR	Linear Amplification PCR And Non-Restricted Linear Amplification PCR
NSG	NOD.Cg-Prkdcscid IL2rgtm1Wjl/Szj (Non-Obese Diabetic [NOD] Severe Combined Immune Deficiency [Scid] Interleukin-2 [IL-2] Receptor Gamma Knockout [Gamma], NSG)
NSGS	NOD.Cg-Prkdcscid IL2rgtm1Wjl Tg(CMVIL3, CSF2,KITLG)1Eav/Mloyszj (NSG-Supportive, NSGS) (Transgenic Expression Of Expression Of Myelosupportive Human Cytokines IL-3, GM CSF And SF)
OD	Optical Density
PB %ALDP+ Cells	Percentage of Cells Expressing ALDP In Peripheral Blood Cells
PB VCN	Peripheral Blood Vector Copy Number
PBL	Peripheral Blood Leukocyte
PD	Pharmacodynamic
PDCO	Paediatric Committee
PE	Platelet Engraftment
PERT	Product-Enhanced Reverse Transcriptase
PIP	Paediatric Investigational Plan
PK	Pharmacokinetics
PRIME	Priority Medicines
qPCR	Quantitative Polymerase Chain Reaction
RCL	Replication Competent Lentivirus
RNaseP	Ribonuclease P
SAE	Serious Adverse Event
SD	Standard Deviation
S-EPTS/LM-PCR	Shearing Extension Primer Tag Selection Ligation-Mediated Polymerase Chain Reaction
SFC- MS/MS	Supercritical Fluid Chromatography with Tandem Mass Spectrometry Detection
SIN	Self-Inactivating
SOC	System Order Class
SUSAR	Suspected, Unexpected, Serious Adverse Reaction
TP	Transplant Population

TPES	Strictly 102 Eligible Transplant Population
TEAE	Treatment-Emergent Adverse Event
TESAE	Treatment-Emergent Serious Adverse Event
TRM	Transplant-Related Mortality
VCN	Vector Copy Number
VSV-G	Vesicular Stomatitis Virus
VLCFA	Very Long-Chain Fatty Acids
VSV-G	Vesicular Stomatitis Virus Glycoprotein

1. Background information on the procedure

1.1. Submission of the dossier

The applicant bluebird bio (Netherlands) B.V submitted on 10 September 2020 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Skysona, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

Skysona, was designated as an orphan medicinal product EU/3/12/1003 on 6 June 2012. Skysona was designated as an orphan medicinal product in the following indication: Treatment of adrenoleukodystrophy.

Skysona was granted eligibility to PRIME on 26 July 2018 in the following indication: for the treatment of cerebral adrenoleukodystrophy.

Eligibility to PRIME was granted at the time in view of the following:

- CALD is a serious and life-threatening condition. The only therapeutic intervention currently available to CALD patients is allo-HSCT for about less than 30% of patients who have access to this donor cell source. Allo HSCT is associated with significant transplant-related morbidity and mortality, including increased risk for graft failure, graft-versus-host disease. Thus, the unmet medical need is agreed.
- Skysona will target patients currently not eligible for allo-HSCT therapy. Preliminary clinical data from 17 treated patients show that the 24 months major functional disabilities (MFD) free survival is 88.2% compared with data from 22 patients treated with allo-HSCT showing a 24-month MFD-free survival rate of 71%.
- The magnitude of the effect could be larger than allo-HSCT and the possibility to treat patients not eligible for allo-HSCT will offer an additional therapeutic option for these patients currently not eligible for allo-HSCT due to the absence of donors.

The applicant applied for the following indication:

Skysona is indicated for the treatment of patients less than 18 years of age with an *ABCD1* genetic mutation and early cerebral adrenoleukodystrophy for whom a human leukocyte antigen (HLA)-matched sibling haematopoietic stem cell (HSC) donor is not available.

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).

Following the CAT/CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Skysona 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/Skysona

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0290/2018 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0290/2018 was completed.

The PDCO issued an opinion on compliance for the PIP P/0290/2018

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.

Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

New active Substance status

The applicant requested the active substance elivaldogene autotemcel contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

PRIME support

Upon granting of eligibility to PRIME, Lisbeth Barkholt was appointed by the CAT as rapporteur.

A kick-off meeting was held on 10 December 2018. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

Post-approval evidence generation including design of a long-term observational registry study

Protocol assistance

The applicant received the following Protocol assistance on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
19 May 2011	EMEA/H/SA/2080/1/2011/ADT/SME/III	*Dr Jan Mueller-Berghaus and Dr Gopalan Narayanan*
20 November 2014	EMEA/H/SA/2080/1/2014/PA/PA/PED/SME/ADT/I	*Dr Jens Reinhardt and Dr Armando Magrelli*
25 February 2016	EMEA/H/SA/2080/2/2015/PA/SME/ADT/III	*Prof. Fernando de Andrés Trelles and Dr Armando Magrelli*
15 and 22 February 2018	EMEA/H/SA/2080/2/FU/1/2018/PA/ADT/PED/II	*Dr Jan Mueller-Berghaus, Dr Mario Miguel Rosa and Dr Armando Magrelli*
15 November 2018	EMEA/H/SA/2080/1/FU/2/2018/PA/ADT/	*Dr Carin Bergquist and Dr Rune*

	PR/I	*Kjeken*
26 March 2020	EMEA/H/SA2080/2/FU/2020/PA/PED/ADT /PR/II	*Prof Livia Puljak and Dr Carin Bergquist*

The Protocol assistance pertained to the following quality, non-clinical, and clinical aspects:

Quality:

- Analytical methods and specifications for the release of the lentiviral vector and finished product.
- RCL testing.
- Commercial manufacturing strategy for patient cell transduction.
- Testing of the cell banks used for lentiviral vector manufacture.
- Manufacturing, characterisation and control strategy for the plasmids used to manufacture the lentiviral vector.
- Manufacturing, characterisation and validation strategy for the lentiviral vector.
- Procurement process and control of the autologous cells.
- Manufacturing, characterisation and validation strategy for the finished product.
- Suitability of the finished product manufacturer.
- Comparability and composition of the finished product.
- Suitability of the potency assay.
- Batch release testing strategy.
- Comparability between finished product manufacturing sites.
- Strategy for stability testing.

Non-clinical:

- Acceptability of the *in vitro* studies for MAA, including vector selection process, the efficiency of transduction and protein expression, and demonstration of biochemical activity.
- Acceptability of the GLP *in vivo* studies for MAA, including the design of the acute toxicology and biodistribution study (duration and endpoints, choice of animal species, single dose).
- Sufficiency of the overall nonclinical studies to support a MAA for the treatment of children and adolescents with CCALD.

Clinical:

- Acceptability of the proposed Phase 2/3 clinical trial design, in particular the trial size, single arm design, duration and scope of follow-up for MAA.
- Acceptability of the proposed functional primary efficacy endpoints and that no overall survival is used as the primary endpoint.
- Acceptability of the safety endpoints.
- Proposed data package and the scientific rationale to seek MAA for the treatment of children and adolescents with CCALD under exceptional circumstances.
- Adequacy of the Study ALD-102 design as a single pivotal study to support MAA for the treatment of children and adolescents with CCALD who present with radiologic evidence of active cerebral disease.
- Acceptability of the statistical analysis plan to describe the analysis of results from Study ALD-102 in comparison to the results from Studies ALD-101 and ALD-103 for MAA.
- Adequacy of the Study LTF-304 design to provide long-term follow-up data on safety and efficacy from subjects with CCALD who have been treated with the product in Study ALD-102.
- Acceptability of the proposed statistical analyses of the primary efficacy endpoint for Study ALD-102, including the criteria for treatment failure and the proposed analyses of the primary safety endpoint.

- Acceptability of the proposed statistical analyses for the secondary efficacy endpoints, including the planned comparison to Studies ALD-103 and ALD 101 for MAA.
- If the proposed statistical analyses for the main secondary safety endpoints support the clinical safety of the medicinal product.
- Acceptability of the main efficacy and safety statistical analyses planned in Study LTF 304.
- If the Agency agrees that the proposed registry study is adequate to monitor long term efficacy and safety of the medicinal product in the proposed patient population in a post marketing setting and if the proposed pharmacovigilance plan and risk minimisation measures are adequate to address the potential safety concerns.
- Agreement with the overall approach to generate real-world clinical evidence post-approval.
- Agreement with the applicant's proposal to use EBMT registry as the main data source for Study REG-502 and the design this post-approval registry study.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Lisbeth Barkholt Co-Rapporteur: Anne Pastoft

The application was received by the EMA on	10 September 2020
Accelerated Assessment procedure was agreed-upon by CAT and CHMP on	23 July 2020
The procedure started on	1 October 2020
The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	22 December 2020
The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	22 December 2020
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	4 January 2021
The PRAC Rapporteur's updated Assessment Report was circulated to all PRAC members on	14 January 2021
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT/CHMP during the meeting on	14 January 2021
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 January 2021
The applicant submitted the responses to the CAT consolidated List of Questions on	25 February 2021
The Rapporteurs circulated the Joint CAT and PRAC Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	31 March 2021
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT/CHMP during the meeting on	09 April 2021
The Rapporteurs circulated the updated Joint CAT and PRAC Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	13 April 2021

The CAT agreed on a list of outstanding issues in writing to be sent to the applicant on	16 April 2021
The accelerated Assessment procedure was reverted to standard timetable	16 April 2021
The applicant submitted the responses to the CAT List of Outstanding Issues on	20 April 2021
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	03 May 2021
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 Skysona on	12 May 2021
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 Skysona on	20 May 2021

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Adrenoleukodystrophy (ALD) is a rare, X-linked, metabolic disease in which dysfunction or lack of the ALD protein (ALDP) is caused by mutations in the ATP-binding cassette, subfamily D member 1 (*ABCD1*) gene. ALDP is a peroxisomal transport protein involved in the transport and degradation of very long chain fatty acids (VLCFA), leading to accumulation of VLCFAs, which occurs most prominently in the adrenal cortex and white matter of the brain and spinal cord.

Cerebral ALD (CALD) is the most severe form of ALD, affecting approximately 40% of boys with ALD, typically during childhood. CALD is characterised by rapidly progressive cerebral demyelination leading to progressive, irreversible loss of neurologic function. If untreated, nearly half of patients with CALD die within 5 years of symptom onset.

2.1.2. Epidemiology and screening tools

The worldwide incidence of ALD among newborn males has been estimated to be approximately 1 in 21,000 among live births, but incidences between 1:17,000 to 1:100,000 have been reported (Wiesinger et al., 2015). There appears to be no major differences in incidence rates of ALD between countries around the world. There is no genotype-phenotype correlation, i.e. whether a newborn child genetically diagnosed with a mutated *ABCD1* gene will develop the aggressive form cerebral ALD during childhood (normally between 2-12 years of age) or only milder disease in adult age cannot be determined based on the type of gene mutation.

The 2 most common neurologic ALD phenotypes manifestations are childhood cerebral adrenoleukodystrophy (CALD) and adrenomyeloneuropathy (AMN), which together account for approximately 80% of all cases. AMN is characterised by slowly progressive predominantly axonal degeneration and affects the spinal cord and to a lesser extent peripheral nerves and typically develop during adulthood. In contrast, childhood CALD is characterised by rapidly progressive cerebral demyelination, resulting in death or vegetative state in most patients within 3 years of onset.

Newborn screening programmes for ALD are underway in several countries; the Minister of Health in the Netherlands has approved the addition of adrenoleukodystrophy to the newborn screening programme. In the USA, ALD was added to the recommended uniform screening panel in 2016.

For boys with a genetic diagnosis of ALD, monitoring of white matter involvement by magnetic resonance imaging (MRI) of the brain is used to objectively determine the onset of CALD signs and identify patients at high risk for rapid progression (i.e. the subgroup of 40% who develop CALD). When signs of CALD have developed, the patient should be treated.

2.1.3. Aetiology and pathogenesis

It is not known why 40% of boys with a genetic diagnosis will develop the rapidly progressive cerebral demyelination form of ALD, the cerebral ALD (CALD). There is no genotype-phenotype correlation. However, in some cases, head trauma appears to have been some kind of trigger, preceding the development of neuroinflammation and cerebral ALD.

2.1.4. Clinical presentation

Disease progression and neurologic decline in patients with CALD have been documented in natural history studies; patients experience critical deficits across multiple neurological domains within a short period after onset of clinical symptoms. In children diagnosed with CALD, learning and behavioural problems are often observed at the time of disease onset in early- to mid-childhood (median age 7 years) with progressive gait, vision, and hearing impairments within 6 to 15 months of symptom onset. This is typically followed by rapid neurologic functional decline.

2.1.5. Management

There is currently no medicine approved for treatment of CALD.

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is a therapeutic option that has been used for a few decades and that can stabilize progression of CALD. The best outcomes are observed if it is performed at early stages of cerebral involvement.

Allo-HSCT is thought to enable migration of donor-derived cells into the brain, which include donor-derived macrophages and/or microglial cells that express functional ALDP and function normally, thereby stopping further demyelination. Although allo-HSCT has been reported to stabilize CALD, the full effects of transplant are not immediately apparent; demyelinating lesions usually continue to progress for 12 to 18 months post-transplant with some clinical progression observed. No effect has been seen on other disease manifestations of ALD, such as adrenal insufficiency, and the slowly developing spinal and peripheral nerves neuropathy in adulthood. It is anticipated that Skysona will only have beneficial effects on the cerebral inflammation part of the disease, just as has been observed for allo-HSCT treatment.

Allo-HSCT procedure also involves a pre-transplant myeloablative and lymphocyte-depleting conditioning regimen. In addition, because the cells received are allogenic, not autologous, the patient may also receive immunosuppressive therapy for several months and sometimes for years after transplant, depending on the degree of incompatibility between the host and donor cells.

Allo-HSCT has potential complications, including risks for transplant-related mortality, graft failure, graft-versus-host disease, and infection. These risks are decreased in patients who have a human leukocyte antigen-matched sibling donor; which fewer than 30% of patients have. Current treatment options for those without an HLA-matched sibling donor include allo-HSCT with cells derived from an HLA-mismatched related donor or from a matched or mismatched unrelated donor, including banked

cord blood. For those receiving alternative donor source cells, the risk of acute or chronic graft versus host disease is higher from an unrelated donor (50.0% for matched unrelated donor and 81.5% for unmatched unrelated donor) versus 25.0% for those receiving cells from a matched related donor.

There is a high unmet medical need for efficacious therapies for CALD that have an improved safety profile compared to allo-HSCT, especially for those who do not have a matched sibling donor.

About the product

Skysona drug product consists of an autologous CD34+ cell-enriched population containing cells transduced with lentiviral vector that encodes an *ABCD1* cDNA for human ALDP. This is classified as an ATMP, more specifically a gene therapy.

Peripheral blood mononuclear cells are collected by apheresis from each patient following mobilisation.

The autologous haematopoietic progenitor cells obtained by apheresis are enriched for cells expressing CD34, and the CD34+ cells are stimulated ex vivo with a mixture of recombinant human cytokines to facilitate cell growth. Next, the cells are transduced with Lenti-D lentiviral vector. After transduction, the cells are washed, resuspended in the cryopreservation solution, and filled in bags before controlled freezing to -140°C. On the day of infusion, the eli-cel drug product is thawed and infused intravenously as a single dose without additional processing steps at the clinical site.

The minimum recommended dose of Skysona is 5×10^6 CD34+ cells/kg. If the minimum dose of Skysona 5×10^6 CD34+ cells/kg is not met after initial medicinal product manufacturing, the patient may undergo one or more additional cycles of mobilisation and apheresis, separated by at least 14 days, in order to obtain more cells for additional manufacture.

Type of application and aspects on development

Accelerated assessment

The CHMP and CAT agreed with the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on the following:

CALD is a rare, rapidly progressive fatal genetic disease with the only disease-modifying available treatment being allo-HSCT. The standard therapeutic method (allo-HSCT) is effective but is associated with mortality and morbidity related to GVHD and graft failure, especially in those patients for whom an HLA-matched donor cannot be found. In addition, there is a need for life-long immunosuppression after allo-HSCT. Since the CALD disease progression is rapid once signs have developed, it is also important that a suitable donor is identified promptly. Children with CALD for whom an HLA-matching is not available (70% of all CALD cases) have an imminent unmet medical need. It is agreed with the applicant that Skysona has the potential to address this unmet medical need and to bring a therapeutic advantage to patients with CALD. With the use of the patient's own HSCs, the search for a suitable donor can be skipped, which saves valuable time for the patient with this rapidly progressive disease. Thus, the applicant's view that this new substance constitutes a major interest from the point of view of public health is supported.

Skysona represents a major therapeutic innovation with a new mechanism of action, i.e. autologous haematopoietic cells transduced with a lenti-viral vector containing the *ABCD1* gene cDNA. Following engraftment, these cells migrate into the CNS where they differentiate into macrophages and cerebral microglia which produce functional human adrenoleukodystrophy protein (ALDP).

Skysona may alter the outcome of untreated CALD and arrest disease progression in a way similar to that observed with allo-HSCT, but with the safety advantage of a lower risk of GVHD or graft failures.

However, during assessment the CAT and CHMP reverted the evaluation to a standard timetable, as although all major objections had been solved, clarifications were still needed at Day150. With regards to the quality dossier, the pending points pertained to tightening of specification limits and confirmation of contractual agreements to ensure traceability throughout the albumin supply chains. In relation to the clinical dossier, the pending clarifications referred to the indication wording, variability of outcomes, the potential risk of insertional oncogenesis and the finalisation of the Product Information.

2.2. Quality aspects

2.2.1. Introduction

The finished product Skysona (also referred to as eli-cel or Lenti-D) is presented as a dispersion for infusion containing 2 to 30 x 10^6 cells/ml of an autologous CD34+ cell-enriched population that contains haematopoietic stem cells transduced with a lentiviral vector (LVV) encoding *ABCD1* cDNA for human adrenoleukodystrophy protein (ALDP) as active substance. The cells are suspended in Cryostore CS5 cryopreservation solution containing 5% dimethyl sulfoxide (DMSO). The product is available in 20 ml fluorinated ethylene propylene (FEP) infusion bag(s), each packed in a transparent pouch inside a metal cassette. One lot of finished product may be packaged in either one or two 20 mL bags, depending on the total number of cells present. Multiple lots may be administered to the patient as a single dose.

Although this dossier is not considered a Quality by Design application, certain elements of an enhanced approached were applied.

2.2.2. Active Substance

The active substance consists of autologous CD34+ cell-enriched population that contains haematopoietic stem cells transduced with a lentiviral vector (LVV) encoding ABCD1 cDNA and is produced from two starting materials, the lentiviral vector (referred to as Lenti-D LVV) and haematopoietic progenitor cells obtained by apheresis (HPC-A).

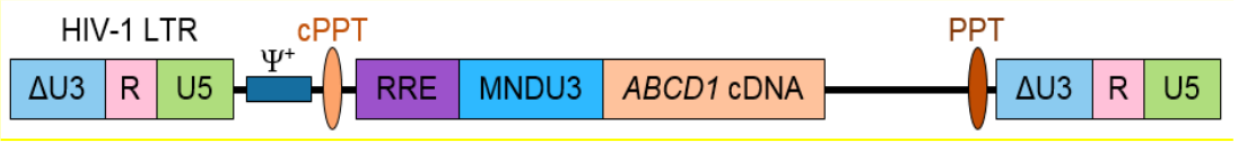
The section on the active substance is separated into two parts; part 1 for the lentiviral vector and part 2 for the transduced autologous cells. The active substance (transduced autologous cells) is formulated directly into the finished product in a continuous process, by formulation in the cryopreservation solution and filling into cryopreservation bags.

Part 1: Lentiviral vector

General information (lentiviral vector)

The Lenti-D LVV is a replication-incompetent, self-inactivating (SIN), HIV-1-based LVV pseudotyped with the envelope glycoprotein G from the vesicular stomatitis virus. The function of Lenti-D LVV is to transfer the RNA genome into CD34+ cells. Viral enzymes reverse transcribes the viral RNA into DNA and integrate the proviral DNA carrying the human adrenoleukodystrophy (*ABCD1*) gene into the CD34+ cell genome. Due to the absence of viral genes in the LVV, the integrated proviral form is incapable of replication.

Figure 1: Provirus sequence Map



From left to right: ΔU3, R and U5 segments; ΔU3 = delta unique3 region, containing a deletion of the U3 enhancer/promoter; R = repeat; U5 = unique 5 region; Ψ+ = extended psi packaging signal containing two STOP codons (not shown); cPPT = central polypurine tract; RRE = Rev response element; MNDU3 = murine myeloproliferative sarcoma virus (MPSV) U3 enhancer/promoter region with negative control region (NCR) deletion; ABCD1 = ATP-binding cassette subfamily D member 1 transgene, encoding the adrenoleukodystrophy protein (ALDP) cDNA (complementary DNA); PPT = polypurine tract; ΔU3 = U3 enhancer/promoter region with negative control region (NCR) deletion; R = repeat;U5 = unique5 region

Manufacture, process controls and characterisation (lentiviral vector)

Description of manufacturing process and process controls (lentiviral vector)

Adequate information on areas of responsibility for manufacturing and test sites for Lenti-D LVV has been provided as required, including confirmation that necessary control measures are in place at bluebird bio, which ensure that the manufacturers of starting materials adhere to the principles of GMP.

In brief, the manufacturing process comprises thawing of HEK293T cells from one vial of the WCB, expansion of HEK293T cells to reach the intended viable cell number for the transfection step, preparation of the transfection cocktail, transfection and LVV production, harvest and purification steps, filtration and filling.

For each process step, critical process parameters (CPPs), non-CPPs, and in-process controls (IPCs) are provided. The CPPs and non-CPPs are controlled within specified ranges.

The information provided on the manufacturing process, process parameters, and process controls for Lenti-D LVV is sufficient.

Lenti-D LVV vials are stored frozen. After release, the vials may be shipped to a long-term storage facility or to an active substance manufacturing site under controlled temperature conditions. Temperature monitoring is performed during transit of all Lenti-D LVV shipments.

Control of materials (lentiviral vector)

The materials of biological origin used in the Lenti-D LVV manufacturing process (other than plasmids and HEK293T cell banks, see below) are described. Materials of biological origin are accepted for use in manufacturing based on successful inspection of the supplier's certificate of analysis (CoA). Information demonstrating that materials meet standards appropriate for their intended use, including source, manufacture, and characterisation are provided and deemed acceptable for their purposes.

HEK293T Cells

The modified, human, embryonic, kidney cell-line, designated HEK293T, is the mammalian packaging cell line used as the cell substrate in the Lenti-D LVV transfection process. Information on the source, history and generation of the cell line is provided.

A master cell bank (MCB) and WCBs of HEK293T cells have been established under GMP and in accordance with Ph.Eur. 5.2.3 and ICH Q5D. Information on the manufacture and qualification of the MCB and WCBs is provided.

WCBs were tested in accordance with ICH Guideline Q5D with regards to identity, viability, and absence of viral and non-viral contaminants. Brief but adequate descriptions of the analytical methods applied for qualification of the cell banks are provided. All test results met specification and the WCBs

were shown to be free of contamination by bacteria, fungi, mycoplasma, and virus as summarised in the dossier. The results support their use in the manufacturing of the LVV.

Assessment of adventitious agents is done through test methods utilising *in vitro* and *in vivo* techniques. Foetal bovine serum is tested by PCR for absence of adventitious viruses in accordance with CFR 9 (Code of Federal Regulations, USA) prior to gamma-irradiation, which is deemed an acceptable approach.

No significant trends were identified over the *in vitro* age range with respect to cell viability, total viable cells, population doubling time, or cell morphology. These results demonstrate the stability of the cell bank system with respect to manufacturing consistency across the *in vitro* age range used in manufacturing. Also, the stability and performance of HEK293T WCBs are monitored for each Lenti-D LVV manufacturing run.

HEK293T cells were expanded during an engineering run and collected prior to transfection to generate an end of production cell bank (EOPCB). Testing in accordance with ICH Q5D demonstrated that the HEK293T EOPCB was free of detectable bacteria, fungi and adventitious viruses and support the use of the WCB.

Plasmids

A multi-plasmid system, consisting of a transfer plasmid (pLBP100) that carries the *ABCD1* (ATP-binding cassette, sub-family D, member 1) gene, which encodes the human adrenoleukodystrophy protein (ALDP), and packaging plasmids is used to manufacture Lenti-D LVV by transient transfection of HEK293T cells. Expression of the *ABCD1* transgene is controlled by an internal enhancer/promoter derived from the unique 3' region of murine myeloproliferative sarcoma virus (MPSV) with a negative control region deletion (i.e., an internal MNDU3 enhancer/promoter). Information on the structural elements, associated plasmid maps and full sequence information for the plasmids is provided.

The plasmid production site is qualified and managed by the applicant, in accordance with their quality management system and the plasmids are manufactured according to the applicant's specifications. Adherence to principles of GMP is ensured by the quality system in place, including audits being performed regularly.

A flow diagram and brief narrative description of the plasmid manufacturing process have been provided, including information of process parameters and in-process controls. Individual WCBs are used for the manufacture of each of the plasmids. The manufacturing process consists of fermentation and harvest, a downstream purification process, ending up with final filtration, filling and storage. Stability has been verified for the proposed shelf-life.

The manufacturing process performance has been evaluated with regards to process control and consistency, aseptic manufacturing, sterilising grade filter validation, cleaning validation, and shipping validation.

The information provided on the manufacturer and the manufacturing process is considered adequate.

Certificates of Analysis have been provided for all cell banks. The generation and qualification of the cell banks, including master, working and end of production cell banks, is in accordance with the requirements of Ph. Eur. 5.14.

The release specifications are accordance with Ph. Eur. 5.14. Brief, but sufficient descriptions have been provided of the analytical methods and the requirements set for validity of the results obtained. A validation summary has been provided, which indicates that all analytical methods have been sufficiently validated (non-compendial) or verified (compendial). The information provided is considered sufficient.

Control of Critical Steps and Intermediates (lentiviral vector)

Please refer to the process characterisation section below.

Process Validation (lentiviral vector)

Under a prospective validation protocol, the Lenti-D LVV commercial manufacturing process was validated by demonstrating that the process performed consistently and met pre-established acceptance criteria, when executed within defined operating ranges. Process performance qualification (PPQ) runs were successfully completed within the normal CPP and non-CPP operating ranges (NORs). All results met the acceptance criteria.

In the context of aseptic filling for LVV manufacturing, data show that no vials in any media fill simulation were positive for microbial growth.

Process characterisation data and classification of process parameters and establishment of proven acceptable ranges (PAR) are presented. The results have laid the basis for the commercial process control strategy. The relationship between unit operation parameters and quality attributes is presented as well as the location of IPCs. Furthermore, the final LVV testing as part of lot release and product characterisation testing is also described. The strategy deciding criticality of quality attributes, as presented by the applicant, is deemed relevant.

The Lenti-D LVV transport is performed using refrigerated shipping systems. Shipping runs (runs with minimal load and maximal load for each shipping system size), were performed under-estimated worst-case conditions with regards to duration (distance) and outside temperature. For all runs the acceptance criteria of product temperature range and time to reach target temperature were met. No damage to packaging system or load was observed.

Overall, the validation studies performed are considered appropriate and the process, aseptic performance, sterilising-grade filter, and shipping conditions are considered successfully validated.

Manufacturing Process Development (lentiviral vector)

The Lenti-D LVV manufacturing process was developed at bluebird bio and transferred. The same manufacturing process has been used throughout the development although minor improvements were made as additional knowledge was gained. The results are in support of the changes and deemed acceptable.

Process characterisation

Process parameters subjected to process characterisation studies were selected through Failure Modes and Effect (FMEA) based risk ranking from evaluation of their potential impact on the identified critical quality attributes (CQAs) and non-CQAs.

Overall, the procedure used for process parameter classification is considered scientifically sound and appropriate and the process characterisation studies conducted are considered adequate. The justifications provided for the established process parameters classifications and the corresponding PARs are considered scientifically sound and acceptable.

Control strategy

Relevant CQAs have been established for the Lenti-D LVV process and ensures Lenti-D LVV of the intended quality. The quality attributes classified as critical are all addressed in the release specifications. The justifications given for the selection of control elements made and the overall control strategy in place are considered appropriate and capable of ensuring a consistent manufacture of Lenti-D LVV product of the intended quality.

Characterisation (lentiviral vector)

Structure

Lenti-D LVV batches were subjected to characterisation with regards to structure in terms of viral protein profiling and detection by capillary electrophoretic (CE) separation followed by chemiluminescent detection of total proteins or identification through detection using virus protein specific antibodies. The identity of the viral proteins necessary to form functional LVV and their presence throughout process development, have been confirmed.

- particle size distribution: Overall, consistent particle sizes within the expected size of a lentivirus were observed.

- total nanoparticle tracking analysis: Overall, consistent number of nanoparticles/mL were observed.

Biological activity

The biological/functional activity of the Lenti-D LVV is characterised through demonstration of transduction efficiency. Batches of Lenti-D LVV have been subjected to characterisation of the biological/functional activity, including batches used for clinical manufacture and the PPQ batches manufactured.

The determination of structure and biological activity is considered adequate and in accordance with EU guidance.

Impurities

Lenti-D product- and process- related impurities are presented, and relevant controls are included in the release specifications. The information on process-related impurities identifies whether each impurity is monitored during release testing or evaluated during characterisation studies of the Lenti-D LVV or manufacturing process. The ability of the Lenti-D LVV manufacturing process to reduce the levels of the impurities was evaluated during process development.

The Lenti-D LVV manufacturing process carries the theoretical risk for generation of RCL. This risk is mitigated by the design of the plasmids used to transfect the HEK 293T cells. The absence of RCL is confirmed through release testing. No evidence of RCL generation during LVV manufacturing using this plasmid system has been observed to date.

The impurities potentially present in Lenti-D LVV are considered adequately characterised. The control strategy in terms of reduction and determination of residual levels of impurities is considered well justified.

Specification (lentiviral vector)

The release and stability specifications for the Lenti-D LVV cover potency, identity, safety, purity, appearance, and quality. In general, the justifications provided for the Lenti-D LVV specifications are considered scientifically sound, adequate and acceptable.

Analytical methods (lentiviral vector)

Summary descriptions of the analytical procedures used for release and stability testing are provided. The descriptions are considered sufficiently detailed and the methods applied fit for the purpose.

Adequate descriptions of the validation or verification (for compendial methods) studies and results have been provided, demonstrating that the validation has been conducted in accordance with ICH Q2.

Batch analysis (lentiviral vector)

Batches of Lenti-D LVV, including PPQ batches, were used to establish the commercial specification. Overall, the release data indicate consistent quality of the Lenti-D LVV batches manufactured throughout process development.

Reference materials (lentiviral vector)

The reference standard has been prepared from a Lenti-D-LVV batch manufactured using the intended commercial manufacturing process. Based on the data provided, the primary Lenti-D LVV reference standard is considered sufficiently qualified and fit for purpose.

As required, a stability protocol and a description of the strategy for introducing a new reference standard have been included. Both are considered acceptable.

Container Closure System (lentiviral vector)

Lenti-D LVV is filled into vials that are stoppered with elastomeric stoppers and capped. The vial and stopper are the Lenti-D LVV contact portions of the container and both are of compendial quality.

An extractable study was performed to generate an extractable profile for the vial and stopper when in contact with the final formulation of Lenti-D LVV. The study design evaluated extracted compounds and elements from simulated worst-case process conditions. Based on the extractable testing performed on the vial, stopper and the container closure system it is concluded that the configuration is appropriate for the intended use.

The container closure system is considered fit for the current purpose.

Stability (lentiviral vector)

Lenti-D LVV batches have been placed on stability, including the PPQ batches, at the intended long-term storage condition to demonstrate the biological and physical stability through the proposed shelf-life. The containers used for stability studies are identical to those used under routine manufacture. All long-term, real-time stability data comply with the specifications in place at the time of testing. Lenti-D LVV batches have been stored and remain within specification, thus supporting the proposed shelf-life. The long-term stability studies are ongoing. Accelerated stability studies are completed and data remain within specification for the duration of the studies. The proposed Lenti-D LVV shelf-life, using the container closure system described, is supported by the stability data provided and is acceptable.

A commitment has been given to continue the stability studies post-approval and to report any confirmed OOS result to the relevant authorities.

Part 2: transduced autologous cells

General Information (transduced autologous cells)

The active substance (INN: elivaldogene autotemcel) is a genetically modified autologous CD34+ cell-enriched population that contains haematopoietic stem cells (HSCs) transduced with LVV encoding *ABCD1* cDNA for human adrenoleukodystrophy protein.

Cells transduced with Lenti-D LVV contain integrated copies of the *ABCD1* transgene, encoding the functional human ALDP which is a peroxisomal membrane protein involved in the transport and metabolism of very long-chain fatty acids (VLCFA).

Eli-cel active substance is identical to the finished product, except that the finished product is resuspended in cryopreservation solution in the final immediate container for the intended medical use.

The eli-cel finished product is intended for treatment of patients with cerebral adrenoleukodystrophy (CALD), derived from mutations in the *ABDC1* gene, which result in diminished or absent ALDP expression and/or function and subsequent accumulation of VLCFAs within multiple tissues. Within the central nervous system and brain, this accumulation of VLCFAs is associated with inflammation and neurodegeneration. Thus, the desired mode of action of eli-cel is to replace the dysfunctional *ABCD1* gene with the Lenti-D LVV encoded functional one and hereby restore cellular transport and metabolism of VLCFA in CALD patients.

Manufacture, process controls and characterisation (transduced autologous cells)

Active substance manufacturing and in-process testing takes place at Minaris Regenerative Medicine GmbH., Ottobrunn, Germany (previously referred to as apceth). MIA and GMP certificate references are provided.

Description of manufacturing process and process controls (transduced autologous cells)

The manufacture of active substance/finished product is a continuous process. A high-level description of the manufacturing process has been provided including a flow diagram, information on media used, the objective and a short narrative of each process step, and tabular overviews of critical and non-critical process parameters and in-process controls. Briefly, mononuclear cells are separated from platelets and other plasma components in the starting material HPC-A by centrifugation and washing and CD34+ cells are further enriched. After enrichment, first a pre-transduction stimulation is performed and then the cells are transduced with Lenti-D LVV. Cells are then washed and re-suspended, resulting in the active substance.

The information provided for the manufacturing process is considered adequate.

Control of materials (transduced autologous cells)

The starting materials used in the manufacturing process are presented. Starting materials include plasmids and human embryonic kidney 293T (HEK293T) cells which are used to manufacture Lenti-D LVV (details presented above in the lentiviral vector section), the Lenti-D LVV and autologous haematopoietic progenitor cells obtained by apheresis (HPC-A).

HPC-A

Procurement of cell starting material is performed in compliance with European Union (EU) Directive 2004/23/EC and implementing Directives 2006/17/EC, 2006/86/EC (as amended by Directive 2015/565), and 2015/566 (referred to as "Tissue Directives"). The program is additionally designed to comply with EU Directive 2002/98/EC and implementing directives ("Blood Directives"). The collection of autologous cells for commercial manufacture of eli-cel is performed by Qualified Treatment Centres (QTCs), which may include an Apheresis Collection Centre (ACC), a Cell Therapy Lab (CTL) and/or a finished product cryostorage location and infusion centre. QTCs are certified or licensed as either Tissue or Blood Establishments in accordance with national legislation implementing relevant EU Directives. QTCs are additionally qualified by the applicant following a programme sufficiently described in the application and involving apheresis assessment, quality audits, quality agreements, on-site training and a follow-up and maintenance programme. The approach is considered adequate.

Information on donor selection is provided. Donor screening and testing of prospective eli-cel patients is performed for the presence of specific infectious diseases prior to the collection of HPC-A. Infectious disease testing is performed by labs that are appropriately qualified using appropriately certified or approved test kits (CE-marked). Since HPC-A is for autologous use, positive test results may not necessarily prevent its use for the manufacture of eli-cel. In certain cases, additional tests for

infectious disease markers may be performed to rule in or out persisting infection. The infectious disease testing strategy to determine acceptance of HPC-A is sufficiently described and deemed acceptable.

A brief description of the HPC-A collection process is provided. Collection of peripheral blood mononuclear cells (PBMCs) is performed following standard operating procedures. The apheresis collection devices and kits are specified, and CE-declarations of conformity are provided.

The HPC-A from a single mobilisation cycle is used to support production of one batch of finished product. If necessary, an additional mobilisation cycle may be subsequently completed following a pre-defined time interval to collect additional cells that will support manufacture of a supplemental finished product batch to achieve the finished product patient dose.

Characterisation data for HPC-A material used in the manufacturing of batches used in the clinical development (studies ALD-102 and ALD-104) are provided.

The shipping system for HPC-A has been qualified using a risk-based approach through both Operational Qualification (OQ, performed by both the vendor and by the applicant) and Performance Qualification (PQ). In addition, shipping qualification studies were performed for worst-case simulated conditions, including atmospheric condition, shock (dropping) and vibration test regimes posed upon HPC-A samples.

Raw materials

An overview of the raw materials used in the active substance manufacturing process is presented, including representative CoAs or certificates of conformance (CoCs). In general, the provided information demonstrates appropriate quality of the raw materials.

Transfer pack containers and cell culture bags of varying sizes, tubing and cell separation kits used in the active substance production are sterilised and single use. Representative certificates of analysis are provided. Based on a risk assessment, an extractable study was performed for the cell processor kit and the cell culture bag. No safety concern was identified.

Control of critical steps and intermediates (transduced autologous cells)

An overview of the IPCs and their action limits included in the active substance manufacturing process is provided. Furthermore, according to the description of the manufacturing process, additional sampling is included after each step.

The limits of the currently proposed IPCs are considered sufficiently justified.

Process validation

As the manufacturing process of Skysona is continuous, process validation is discussed in the finished product section.

Manufacturing process development (transduced autologous cells)

The description of eli-cel active substance manufacturing process development includes an overview of the manufacturing process history that describes changes introduced during clinical development and compares clinical manufacturing results across multiple manufacturing sites, an overview of the approaches employed for process design and development with justifications for the use of healthy donor cell HPC-A and scale-down models, results from process characterisation studies, and a process control strategy (PCS) summary for the commercial manufacturing process.

Overall, the same manufacturing process has been used throughout the eli-cel active substance manufacturing process history, although minor process improvements were made as additional knowledge was gained.

Comparability

Throughout development, clinical trial materials were manufactured at several sites. During process transfer between sites and upon defining the commercial process, minor sequential adjustments of the process were made. A site-to-site comparison was conducted with eli-cel material from the manufacturing sites used during clinical development. The strategy is considered adequate.

The analytical approach includes a classification of attributes based on their potential impact to product quality, safety or efficacy and corresponding development of acceptance criteria following a risk-based approach. The risk classification is clearly described and considered adequate.

The data provided support comparability between sites.

Process development studies

Process development studies (process characterisation and PPQ) were conducted using surrogate cells. Results are provided to show that data generated with surrogate cells (at-scale engineering run data) can be extrapolated to product obtained from CALD patients. Justification of the use of surrogate cells in development studies is based on a comparison of in process and final product attributes of engineering batches with data from clinical batches. Data provided support the use of surrogate cells as model for patient cells.

A scale-down model was developed to enable design of experiment (DoE) studies for both process development and characterisation studies.

An evaluation of the eli-cel quality attributes was conducted following quality risk management principles. The risk-based approach used to assess the criticality of each quality attribute included an evaluation of the severity of impact and the uncertainty of knowledge with regards to its clinical impact. Quality attributes related to safety, identity, strength, potency, purity, process-related impurities, LVV-related impurities and general attributes have been addressed.

Process parameters were assessed for potential impact to quality attributes. Individual parameters were assigned a risk score based on severity, occurrence and detection, using a Failure Mode and Effect Analysis (FMEA) approach. Parameters with a risk score above a pre-established threshold were designated for further process characterisation. This results in a selection of process parameters for further evaluation using DOE, one factor at a time, or a combination of both approaches at different scales. The provided summaries of the process characterisation, although brief, are considered sufficient to assess the impact of process parameters on study output attributes and consequently to support the proposed parameter classification and PARs.

Characterisation (transduced autologous cells)

Elucidation of structure and other characteristics

Characterisation studies were performed on retains from eli-cel batches used in clinical studies.

Multiple attributes and correlations were analysed including VCN of all cells and colony-forming-cells, the percentage of cells with integrating transgene and the percentage of LVV+ eli-cel colonies, percentage of eli-cel cells expressing ALDP and its functional impact, retention of the capability to form colonies as well as phenotypic cellular composition. State-of-the-art-methods were applied and the results confirmed the expected quality attributes.

VCN

Orthogonal methods were used to evaluate VCN. The theoretical risk of tumorigenesis in colonies with high VCN values for the subpopulations of eli-cel has been sufficiently discussed and the control strategy in place for mitigating this risk is considered adequate.

%LVV+ cells

Orthogonal %LVV+ (percentage of transduced cells) measurements provide similar estimates of the transduction efficiency in both the microglia progenitors and in eli-cel cells that engraft the bone marrow.

%ALDP+ Cells and Potency by VLCFA Reduction

Quantitative flow cytometry is used to determine the percent of cells that express ALDP in both eli-cel retains and in matched, untransduced patient CD34+ cells from the same subject. In all analysed eli-cel batches successful expression of ALDP from the *ABCD1* cDNA was detected.

A substrate reduction characterisation assay is used to investigate whether the expressed ALDP protein is functional.

Correlations between eli-cel attributes and clinical biomarkers

No differential relationship could be established between eli-cel attributes and the clinical outcome as determined by the occurrence of major functional disability (MFD) or Neurologic Function Score (NFS) change from Baseline at Month 24, most probably due to the reduced number of patients developing negative clinical outcomes. Additional clinical biomarkers were included in the characterisation studies to further investigate the relevance of eli-cel attributes for clinical efficacy. The results presented demonstrated correlations between VCN and peripheral blood (PB) VCN at Month 6; VCN and PB %ALDP+ cells at Month 6 as well as between %LVV+ cells and PB %ALDP+ cells at Month 6.

Characterisation of eli-cel colony-forming activity

A colony forming assay is used to investigate whether eli-cel cells are maintaining their ability to form colonies. Data presented indicate that all eli-cel lots were able to form colonies, and that colony-forming capacity of haematopoietic stem and progenitor cells (HSPCs) is not lost during transduction with Lenti-D LVV and manufacturing process.

Impurities

A comprehensive list of the potential process-related impurities together with the steps at which these reagents are introduced in the manufacturing process and the analytical methods used for detection is provided. Their criticality is defined based on the potential risks posed by the presence of these process-related impurities in the active substance. Short descriptions of the methods used for impurity characterisation as well as defined limit of quantitation (LoQ) and/or limit of detection (LoD) for these assays are provided.

Product related impurities have been adequately addressed in terms of CD34- cells, untransduced cells and non-viable cells, which all are controlled in the finished product release specification. Furthermore, extensive deep phenotypic characterisation was performed using different methods, demonstrating that the percentage of other cellular impurities (T-cells, B-cells, monocytes, granulocytes, NK cells) is low.

Specification (transduced autologous cells)

A specification for the active substance is not applicable.

Stability (transduced autologous cells)

Stability testing of active substance is not applicable.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

The finished product is presented as a dispersion for infusion containing 2 to 30 x 10^6 cells/ml of an autologous CD34+ cell-enriched population that contains haematopoietic stem cells transduced with a lentiviral vector (LVV) encoding ABCD1 cDNA for human adrenoleukodystrophy protein (ALDP) as active substance. The cells are suspended in Cryostor CS5 cryopreservation solution containing 5% dimethyl sulfoxide (DMSO). The product is available in 20 ml fluorinated ethylene propylene (FEP) infusion bag(s), each packed in a transparent pouch inside a metal cassette. The composition is shown in Table 1.

Table 1 Finished product composition

Component	Function	Quality Standard	Amount per Batch
Autologous CD34+ cell-enriched population containing cells transduced with lentiviral vector that encodes an *ABCD1* cDNA for human ALDP (eli-cel drug substance)	Drug substance	In-house	2×10^6 - 30×10^6 cells/mL
CryoStor CS5[a]	Excipient for suspension and preservation of cells in ultralow temperature environments	Vendor Certificate of Analysis	20 mL per bag, up to two bags

[a] CryoStor CS5® is a commercially available cryopreservation solution pre-formulated with 5% dimethyl sulfoxide (DMSO)

One lot of finished product may be packaged in either one or two 20 mL bags, depending on the total number of cells present. Multiple lots may be administered to the patient as a single dose as documented in the Lot Information Sheet that accompanies the final product shipment.

Adequate information on the excipient CryoStor CS5 is provided, including composition and specifications. CryoStor CS5 is released for production at the finished product manufacturing site based on visual inspection, inspection of the supplier Certificate of Analysis, and identity testing based on direct injection gas chromatography for determination of DMSO content.

An overview of the manufacturing process development is provided. The finished product manufacturing process, consisting in formulation and cryopreservation, has remained unchanged during development and transferred to several manufacturing sites.

Information on the selection of the container closure system is provided and is considered adequate. The selected container closure system supports product stability and is expected to be suitable for its intended use. The provided information on extractables is deemed sufficient. The applicant has provided a brief discussion of relevant aspects for microbiological safety, including testing of the finished product and the steam sterilised container. Container closure integrity was also tested after freezing, shipping and thawing of the bags and it met its specifications.

Manufacture of the product and process controls

Batch release takes place at Minaris Regenerative Medicine GmbH., Ottobrunn, Germany (previously referred to as apceth). The active substance consists of the washed, transduced CD34+ enriched cells in a transfer pack. The active substance is centrifuged, formulated (i.e., resuspended) in

cryopreservation solution and filled into bags to manufacture the finished product. There are no hold steps between active substance and finished product manufacture.

The finished product manufacturing process consists of formulation and cryopreservation. Several process parameters were characterised to establish criticality, NORs and PARs. Based on data obtained in the characterisation of the manufacturing process relevant process parameter limits are established. Additional relevant parameters have been addressed.

The labelling and traceability system is described, particularly the chain of identity. In general, the description is considered sufficient to ensure that the patient receives the correct product.

Process validation

As the manufacturing process for Skysona is performed in a continuous flow, the active substance and finished product process were qualified in the same PPQ runs. Sufficient information is provided demonstrating that for all investigated critical quality attributes, material is representative for the finished product. Based on additional analysis, indicating a statistical correlation between the proposed functional potency assay and the critical quality attributes currently included in the validation exercise, data provided are considered sufficient to support the conclusion that the manufacturing process is validated also with regards to %VLCFA reduction.

Provided data indicate that LVV variability is probably not a major contributor to eli-cel variability and therefore the proposed approach is deemed acceptable.

During the PPQ runs minor deviations were reported. These deviations are appropriately discussed and give no reason for concern.

Results of IPCs, CPPs, non-CPPs and other operational parameters evaluated during process characterisation were provided for all steps included in the manufacturing process. In general, all acceptance criteria are met. All CPPs and non-CPPs where within their specified NORs with one exception which has been appropriately discussed and gives no reason for concern.

The available batch analysis data for all quality attributes support the consistency of finished product manufacturing at the commercial site.

Aseptic process simulation is performed regularly under conditions that are representative of actual manufacturing conditions. Results have been provided for three consecutive media fill simulations and gave satisfactory results without any contaminant units. Results for the media fill cover the maximum process time for manufacturing of finished product and demonstrates that aseptic conditions are maintained.

Validation of the shipping system was performed. For performance qualification, mock product was shipped to locations representing the extremes of high temperature and distance. The approach is considered adequate.

Product specification

The proposed release specification includes tests for Potency, Identity, Purity, Strength, Safety and Quality.

The proposed tests to control the finished product are acceptable. In general, the acceptance criteria for release are sufficiently justified and supported by batch analysis data.

The strategy for release testing is described, including sampling points for tests performed on process intermediates, description of the matrices and justifications for each sampling point.

The testing panel is in general in line with expectations. The currently proposed acceptance criterion for the potency indicating attribute (% VLCFA reduction) is considered acceptable. However, this limit should be re-evaluated and, if needed, revised based on results obtained at release when a sufficient number of batches have been manufactured (See "Recommendations for future quality development"). During the procedure, a major objection was raised in relation to the proposed potency assay (% VLCFA reduction assay), covering the lack of established specification limits, a request for further justifications to support the stability and validation data packages, a request for further data on the first step in the assay, and a request for data demonstrating the successful transfer of the potency assay to the batch release testing site. Additional data and justifications provided in response were considered acceptable and the major objection is considered resolved, with a recommendation to further review the acceptance criteria (discussed above) and to provide the final transfer validation report.

In response to a major objection raised, the applicant has provided a risk evaluation concerning the presence of nitrosamine impurities. The risk assessment determined that there is no risk of nitrosamine impurities in the finished product. The conclusion is endorsed, and thus the outcome of the nitrosamine risk evaluation is considered acceptable.

Analytical methods

An overview of the analytical procedures is provided. In general, the information is considered adequate. The methods are adequately described, and relevant validation data are provided for the non-compendial methods. Compendial methods have been appropriately verified.

Batch analysis

Batch analysis data presented include the 46 batches manufactured for subjects with cerebral adrenoleukodystrophy (CALD) treated in Studies ALD-102 and ALD-104. These include batches manufactured at the proposed commercial manufacturing site.

Data support the consistency of the finished product manufactured at all sites.

Reference materials

A reference standard is not used in the testing and release of finished product.

Stability of the product

Stability studies have been performed in accordance with current ICH/CHMP guidelines.

Due to the autologous nature of the finished product and the limited number of patient cells, stability studies are performed on finished product manufactured from surrogate donor cells. This approach is considered acceptable.

Long-term, accelerated and stress stability studies were conducted. Similar stability indicating attributes are addressed in all these studies.

The strategy for (indirect) stability testing of the metabolic function is accepted, considering also that the limited amount of material produced for each batch is normally used entirely for treatment of the patient.

Long term stability data are available for several batches including PPQ batches and batches manufactured at the intended commercial manufacturing site. In general, results comply with the specifications.

Overall, the available data support the proposed shelf life of 6 months at ≤-140°C.

Accelerated stability studies performed are completed. Stability attributes remain within respective protocol specification throughout the test periods. No apparent trends were observed.

Stability of batches at 18-25°C was studied and provided data support the proposed post-thaw infusion time of 4 hours.

For the freeze/thaw studies performed on PPQ batches, diluted cryopreserved finished product was removed from the cryopreservation unit, stored and refrozen. Stability attributes for these samples remained within specification limits and similar data were observed, as compared to control (not thawed) cryovials.

In conclusion, the available data support the proposed shelf life of 6 months at ≤-140°C and the proposed handling/administration procedures as outlined in the SmPC.

Adventitious agents

The applicant has provided a general overview of the adventitious agents safety evaluation, including information on the control of materials, control of the manufacturing environment, and the testing for potential adventitious agents.

Non-viral adventitious agents

The information provided on the control of non-viral adventitious agents is considered sufficient.

An overview table is provided of the testing performed for mycoplasma, sterility, bioburden, and endotoxin at the various stages of the manufacturing process. Animal derived materials are discussed with regard to the risk for transmissible spongiform encephalopathy (TSE). Bovine derived materials are sourced from animals in the US, Canada, or New Zealand. TSE certificates of suitability have been provided. The risk for TSE is considered negligible.

Viral adventitious agents

Virus safety is built on adequate control of starting materials and raw materials as no steps capable of inactivating or removing viruses are included in the manufacturing process.

An overview of the adventitious agent testing at various stages of the manufacturing process and at final batch release for starting materials and finished product has been provided.

Donors are tested for infectious agents listed in the EU Cell and Tissue Directive. Summaries are provided on the origin of plasmids and cell banks, as well as an overview of the cell bank (MCB, WCB, EOPC) testing results and method summaries of the tests used for cell bank qualification.

For all materials of human or animal origin assessments of the risk for adventitious virus transmission are performed.

In general, the information on human- and animal-derived materials is in line with relevant guidelines and directives and is evaluated to sufficiently mitigate the risk of adventitious agents' contamination.

2.2.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Two major objections were raised during the procedure. One related to the potency assay and covered the lack of established specification limits, a request for further justifications to support the stability

and validation data packages, a request for further data on the first step in the assay, and a request for data demonstrating the successful transfer of the potency assay to the batch release testing site. The second major objection related to the absence of a risk evaluation concerning the presence of potential nitrosamine impurities. In response, the applicant submitted additional data and justifications and the major objections are now considered satisfactorily resolved.

At the time of the CAT/CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product, which pertain to review of in-process/specification limits based on further manufacturing experience, provision of the final transfer validation reports for the potency assay, further process characterisation, and confirmation of contractual agreements between the raw material suppliers and the finished product manufacturer. These points are put forward and agreed as recommendations for future quality development.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. 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/CHMP recommends points for investigation, pertaining to review of in-process/specification limits based on further manufacturing experience, provision of the final transfer validation reports for the potency assay, further process characterisation, and confirmation of contractual agreements between the raw material suppliers and the finished product manufacturer.

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

2.3. Non-clinical aspects

2.3.1. Pharmacology

Mechanism of action

The Lenti-D LVV drug product, Skysona, is a cell dispersion for infusion. Its active substance consists of an autologous peripheral blood (mPB)-derived CD34+ cell-enriched population from patients with cerebral adrenoleukodystrophy (CALD) that contains CD34+ haematopoietic stem cells (HSCs) transduced ex vivo with the Lenti-D lentiviral vector (LVV) that encodes for the human ATP binding cassette (ABC), subfamily D, member 1 (*ABCD1*) transgene (cDNA) and expresses human adrenoleukodystrophy protein (ALDP). Thus, the Lenti-D LVV is not administered to patients; it is used to transduce the patients' own autologous HSCs ex vivo during the manufacture of the drug substance.

Skysona is intended for autologous use only, as a single IV infusion of 2-30 × 10^6 CD34+ cells/mL dispersion for infusion,(the minimum recommended dose of Skysona is 5 × 10^6 CD34+ cells/kg) to treat patients less than 18 years of age with early CALD for whom a human leukocyte antigen (HLA)-matched sibling haematopoietic stem cell (HSC) donor is not available.

CALD is an X-linked recessive genetic disease caused by a mutation in the ALDP-encoding *ABCD1* gene. ALDP is responsible for transportation of very long chain fatty acids (VLCFA)s and VLCFA-CoA into the peroxisome, where VLCFAs are broken down. In the absence of functional ALDP, VLCFAs

accumulates in tissues throughout the body. The most severely affected cells and/or tissues are those that specialize in fatty acid metabolism: myelinated cells in the white matter of the CNS, adrenocortical cells in the adrenal glands, and interstitial (Leydig) cells within the testes. A prominent manifestation of CALD is the accumulation of VLCFAs in tissues that is associated with progressive demyelination and cerebral inflammation within the brain, which, if untreated, leads to severe loss of neurological function over time, and death. One of the hallmarks of inflammatory disease in CALD is the presence of a compromised BBB behind the leading edge of demyelinating lesions. The compromised BBB is suggested by the applicant to facilitate distribution of the product to the brain, i.e. migration of cells deriving from the autologous transplanted transduced CD34+ mPB HSC across the BBB. ALDP deficiency results in highly heterogeneous disease presentation among subjects and there is limited correlation between genotype (mutation) and phenotype (disease manifestation).

Primary pharmacodynamics *in vitro*

For Lenti-D Drug Product, the primary pharmacodynamic effect was defined as the production of transgenic ALDP, which was represented by the proportion of ALDP-positive (%ALDP+) cells. The secondary pharmacodynamic effect was defined as improving or correcting very long chain fatty acid (VLCFA) metabolism.

Due to difficulties in obtaining the cell population on which the clinical therapy is based on (i.e. CD34+ mPB HSC from CALD patients), the pharmacodynamic studies have been carried out with the use of various surrogate cell populations, as CD34+ mPB HSC from healthy donors, ALDP-defect fibroblasts (FB)s from ALD patients and ALDP-defect CD34+ mPB HSC from AMN patients. In scientific advice received from the CHMP, the surrogate cell strategy was accepted in general terms.

From the comparison of lentiviral vectors expressing the *ABCD1* gene from various promoters the internal MNDU3 promoter was selected for use in Lenti-D LVV, as it was the most efficient transducer of ALD FBs and human healthy donor mPB CD34+ HSCs, as compared to Lenti-D LVVs with internal EFs or PGK promoters. Lenti-D LVV (MNDU3), was also the most efficient promotor for expressing ALDP in ALD donor FBs and healthy human mPB-derived CD34+ HSCs. Moreover, there was a direct relationship between MOI and VCN values and %ALDP+ cells after Lenti-D LVV transduction of human ALDP-deficient FBs with MOI ranging from 0.3 – 5.5.

Transduction of 6 different lots of healthy donor mPB-derived CD34+ cells with Lenti-D LVV (MOI = 90% LVV supernatant) resulted in consistent and comparable results, with minimal (<2-fold) variability, for VCN values and %ALDP+ cells in 7-day liquid or 14-day methylcellulose cultures. A trend for lower colony counts in transduced, as compared to mock-transduced. Lenti-D LVV transduction of ALDP deficient human AMN donor mPB-derived CD34+ cells at increasing MOIs, also resulted in VCN values, %ALDP+ cells and improvements in VLCFA metabolism that were directly related to MOI. VLCFA metabolism was corrected at higher MOIs (≥29; ≥40% LVV supernatant). Also, for the ALDP deficient human AMN donor mPB-derived CD34+ cells, the highest MOI (58; 80% LVV supernatant) was associated with lower colony counts.

Transduction with Lenti-D LVV of Human AMN and healthy donor mPB-derived CD34+ cells conducted by two different operators was validated with generally similar outcome. In addition, and in agreement with the other studies, transduction at higher MOI (in this study, values ≥44) provided only modest increases in VCN values and %ALDP+ cells. The colony counts also appeared to be lower at MOIs ≥44.

Primary pharmacodynamics *in vivo*

There are no animal models of CALD that recapitulate the human disease and could be used for demonstration of improvements in cerebral inflammation and demyelination. For this reason, an *in vitro* bridging strategy was applied by demonstrating preclinical POC for Lenti-D LVV-mediated

correction of VLCFA metabolism using cultured ALD FBs and AMN CD34+ HSCs. In vivo, preclinical POC for brain engraftment of Lenti-D transduced CD34+ HSCs was evaluated in myeloablated immunodeficient mice in pivotal combined *in vivo* pharmacology, single-dose toxicity, genotoxicity and biodistribution studies in immunodeficient (NSG and NSGS) mice.

Bone marrow (BM) engraftment and chimerism were displayed in myeloablated NSG mice, at Month 1 and Month 3 after they had received mock- or Lenti-D LVV-transduced human healthy donor mPB-derived CD34+ HSCs. Similar results were obtained after Lenti-D LVV- as well as mock-transduction. A trend towards higher engraftment in female mice was noted. In the subsequent study, only female mice were used. In addition, there was a reduction in engrafted hCD45+ BM cells from Month 1 to Month 3 of 11 or 34 % in Lenti-D LVV or mock-transduced female mice, respectively. A similar reduction in VCN values, by 29%, from Month 1 to Month 3 was also reported.

Migration to the brain of human-origin haematopoiesis-derived cells (i.e., hCD45+ leukocytes), with a human microglial cell immunophenotype (hIba-1+) and expressing a human microglial cell protein (hFGFR-1+) was demonstrated to occur in myeloablated NSGS mice, subsequently to BM engraftment of transplanted Lenti-D LVV- or mock-transduced human healthy donor mPB-derived CD34+ HSCs. No such migration of human cells could be demonstrated in NSG mice. In the transplanted NSGS mice, it was not possible for the applicant to demonstrate specific staining of hALDP+ cells within the brain, although migration of human CD45+ cells with microglial phenotype was demonstrated.

Secondary pharmacology

No secondary pharmacology studies have been carried out. There was no indication of any safety related findings potentially related to secondary pharmacology in the toxicology part.

Safety pharmacology

No dedicated safety pharmacology studies have been carried out. No overt toxicity in CNS, cardiovascular or respiratory system related to administration of Lenti-D LVV has been observed in the two animal studies.

2.3.2. Pharmacokinetics

Conventional PK studies were not conducted as Lenti-D Drug Product is an autologous human cell-based product. The biodistribution of human Lenti-D LVV transduced CD34+ HSCs from healthy donors were investigated in two different strains of immune-deficient mice (NSG and NSGS). Cellular transduction with Lenti-D LVV is performed ex vivo and no free viral vector should be present in final Drug Product.

It should be noted that the difficulties for mimicking human engraftment in non-clinical studies are acknowledged and due to the species barrier and other factors, the biodistribution results from immune-incompetent mice are most likely not fully translatable for how the cells distribute and persist in humans.

The methods of analysis were not validated. However, they are overall considered to have been conducted with sufficiently high scientific standards and the results are not questioned.

The PK and biodistribution of Lenti-D LVV transduced human CD34+ cells were evaluated in two combined pharmacology, biodistribution and toxicology studies, both in immune-incompetent mice (NSG and NSGS). Both studies used IV injection (clinical route) and busulfan pre-conditioning (used clinically) and investigated biodistribution parameters of mock (no LVV) or Lenti-D LVV transduced CD34+ human cells following a single IV injection. Both studies used a single dose of 40×10^6 cells/kg,

which according to the applicant was the maximal feasible dose based on the number of cells available and cell concentration/allowable dose volumes. The dose represents an 8-fold multiple of the minimum dose of Lenti-D Drug Product for patients with CALD (≥5×10^6 CD34+ cells/kg). The VCN were within the clinical range.

IV administration of Lenti-D LVV-transduced human autologous CD34+ HSCs to myeloablated patients with CALD is hypothesised to result in initial distribution to most tissues via the circulatory system. Indeed PCR analysis detected human DNA sequences (*Hu-hck1*) and Lenti-D viral DNA sequences (*Psi-gag*) in all tissues tested (bone-marrow, brain, heart, intestine, kidney, liver, lung, ovaries, skeletal muscle, spleen and testes) which remained throughout the 3-month study period. For human sequences (*Hu-hck1*) and Lenti-D viral sequences (*Psi-gag*), the highest concentration (copies/µg) was observed in bone-marrow were signals above the upper LoQ were observed. Medium to high concentrations were observed in lung, liver and spleen whereas medium concentrations were detected in heart and kidney. Lower concentrations were seen in brain, intestine and muscle.

Following engraftment in bone-marrow, the cells are believed to differentiate further into various cell types. Also, some HSCs are thought to be able to engraft directly with certain tissues such as the brain. The hypothesised mode of action of Lenti-D Drug Product requires transduced HSCs to differentiate into microglia-like cells expressing ALDP and engraft and persist within the brain. Studies on the distribution of the transduced cells to the brain are therefore regarded key for supporting the proposed mechanism of action. The biodistribution data in immune-incompetent mice are considered to support Lenti-D LVV transduced CD34+ HSC engraftment and persistence in bone-marrow and the brain. It should however be noted that human DNA signals in brain were very low and ALDP production in brain could not be demonstrated.

Overall, these results could lend support to the notion that Lenti-D Drug Product can distribute to brain and persist as microglia-like cells in CALD patients.

Germ line transmission studies

No dedicated germline transmission studies have been conducted. Given that the cells are transduced ex vivo with a replication-incompetent vector and no residual free viral particles are present in the Lenti-D Drug Product, the risk for germ line transmission is considered regarded very limited and rather theoretical. Also, patients will be preconditioned with the reprotoxic busulfan, often resulting in infertility. The omission of specific germ line transmission studies is therefore acceptable.

2.3.3. Toxicology

The non-clinical safety programme conducted with Lenti-D LVV transduced human CD34+ HCSs consists of two single-dose toxicology studies performed in NSG and NSGS immune-incompetent mice. Since Lenti-D LVV will integrate in the genome, the potential for insertional site mutagenesis to induce transformative changes was investigated in murine lineage-negative (Lin-) bone marrow cells (BMCs). Furthermore Lenti-D LVV integration patterns in healthy human CD34+ cells were analysed by integration site analysis (ISA).

No repeat-dose toxicology, dedicated carcinogenicity, juvenile or developmental and reproductive toxicology studies were performed, and their omission is acceptable considering the nature of the product.

Relevance of animal models

Lenti-D LVV is a single-dose autologous cell therapy. If human cells are administered to immune-competent animals, they will be rejected. Therefore immune-incompetent mice strains were used. Since mice (including immune-incompetent mice) do not provide optimal physiological conditions for

promoting survival and function of human cells, the relevance of the non-clinical *in vivo* model, in particular for predicting risk of toxicity and cancer development in humans, is considered limited. NSG mice lack mature T or B cells, functional NK cells, and cytokine signaling and predominately promote development of lymphoid cells. NSGS mice, in comparison to NSG mice, also express human cytokines (IL-3, GM-CSF and SF) promoting differentiation of myeloid cells. Although the value of using immune-incompetent mice to predict safety issues for a human cell therapy is questionable, the strains have been used as a model for HSC engraftment and their use can from that point be justified.

Single-dose toxicity

The toxicity of Lenti-D LVV transduced human CD34+ cells were evaluated in two separate single-dose studies, both in immune-incompetent mice. One study was GLP compliant and used NSG mice and the other study was an investigative study and used both NSG and NSGS mice that received busulfan to induce myeloablation and facilitate HSC engraftment in bone marrow.

In the GLP study (NC-12-007), cells were well-tolerated in NSG mice over the 3-months study period. Comparable engraftment of cells was seen in mock and Lenti-D LVV transduced cells. The toxic effects of busulfan makes it difficult to pinpoint Lenti-D LVV-related toxicity. That given, there were no Lenti-D LVV-related adverse effects on clinical signs, body weights or clinical pathology parameters. A NOAEL of 40×10^6 cells/kg (8X margin) is therefore acceptable.

In the investigative study B1-13-003 using both NSG and NSGS mice, the only toxicological parameters investigated were clinical signs, body weights, haematology, serum chemistry (only phosphorous) and organ weights (brain and tibias only). All animals survived throughout the study but from week 10, cells (both mock and Lenti D cells) were not well-tolerated by NSGS mice and signs of myeloid-driven bone-marrow dysfunction were present (weight loss, abnormal clinical signs, decreased platelets, immature blood cells). This was most likely due to the transgenic expression of human myelosupportive cytokines (IL-3, GM-CSF and SF) in NSGS mice that promotes a biased engraftment and differentiation of myeloid cells and leading to myeloid-driven bone marrow dysfunction.

Again, the Lenti-D LVV transduced cells were overall well-tolerated by NSG mice. Similar to the results from the GLP-study there were no clinical observations. A decrease in body weight change (-30%, 0-3 months) and a slight decrease in body weight was observed in animals receiving Lenti-D LVV transduced cells. The decrease was more pronounced between week 10 and 13 (-52,9%). Platelet counts were decreased in Lenti-D treated animals (-51%). The relevance of the weight and platelet findings are unclear since no clear body weight effects or decreased platelets were seen in the GLP study.

Repeat-dose toxicity

As Lenti-D Drug Product is intended for a single autologous IV administration, not performing any repeat-dose toxicity studies is acceptable.

Genotoxicity

Lenti-D is an autologous human cellular product, therefore, standard genotoxicity studies are not considered relevant. Since Lenti-D LVV will integrate in the genome, the primary safety concern for Lenti-D Drug Product is the risk that it may be associated with an oncogenic event resulting from insertional mutagenesis. The applicant has performed an *in vitro* immortalisation (IVIM) study performed in Lenti-D LVV-transduced mouse lineage-depleted (Lin-) BMCs. Moreover, the integration patterns in human healthy donor CD34+ HSCs, pre and post transplantation into mice, were characterised by integration site analysis (ISA).

In vitro immortalisation (IVIM) study: Although the IVIM assay is routinely used and may provide some information on possible gene deregulations, it should be noted that comes with several limitations with the most obvious being that it is performed in murine cells and non-clinical integration studies are largely not predictive for the integration in treated patients.

Overall, the IVIM results indicate that Lenti-D LVV has a significant reduced risk of *in vitro* immortalisation of murine BMCs compared to positive control vectors.

For the IVIM assay, the BMCs were transduced with Lenti-D LVV. Two weeks after limited dilution plating in 96- plates, wells were evaluated microscopically and metabolically (MTT assay) for an immortalised or transformed phenotype. A fitness score (FS) was calculated by normalising the replating frequency (RF) to VCN values. The IVIM assay included positive control vectors RSF91 (gamma- retroviral vector) and lv-SF (lentiviral vector) previously demonstrated to induce immortalisation. Mean VCN values for Lenti-D LVV cells were 4,78 c/dg (6,06; 6,11; 2,1) which, in two of three experiments, were above the highest VCN used in the clinical studies (3,1).

Data of three independent IVIM assays indicated normal cell proliferation for Lenti-D LVV and the positive control vectors when compared to mock transduced cells (no viral vector).

Evidence of *in vitro* genotoxicity was observed in the positive controls RSF91 (gamma retroviral vector) and Iv-SF (Lentiviral vector) confirming the sensitivity of the assay. Lenti-D LVV cells had a low fitness score ($\times 10^{-4}$) of ~0.1. However, the MTT assay was positive in 33% for Lenti-D LVV compared to 98-100% in the positive controls. Moreover, Lenti-D LVV cells where negative microscopically in two experiments but one experiment showed weak proliferating colony growth in two wells. It is not clear if this clone could be further expanded. Only one single Lot of Lenti-D LVV was tested in the IVIM assay. Optimally, several lots should be tested since the fitness score can vary between IVIM assays and vector lots.

Integration site analysis (ISA): Insertional mutagenesis that could promote oncogenesis is considered a primary safety concern. Lenti-D LVV integration patterns were studied in human CD34+ cells (from healthy donors) pre-transplant (*in vitro*) and post-transplant (*in vivo*, BMCs from myeloablated immunodeficient NSG mice from the NC-12-007 GLP study) by integration site analysis (ISA). LAM-PCR in combination with gel electrophoresis and pyrosequencing was used to determine the integration pattern and data were compared to a random data set of 4742 insertional sites (IS).

Overall, the results are consistent with previously described LVV integration profiles with preferred integration in gene-coding regions across the whole genome with no preference for integration in transcriptional start sites and no bias for the 5´or 3´end of genes.

Gel electrophoresis images of LAM-PCR amplicons indicated that pre-transplant (*in vitro*) transduced cells had a polyclonal pattern whereas an oligoclonal/polyclonal pattern was seen in post-transplant (*in vivo*) BMCs collected after 1- or 3-months. No obvious difference in clonal size of BMCs harvested at 30 days and 90 days was detected, indicating that no clonal selection occurred *in vivo.*

The chromosomal distribution of all IS appeared to be close to random but a preference for integration on chromosomes 17 and 19 was observed. An enrichment of IS was seen within gene coding regions compared to regulatory elements with the overall frequency of IS within gene-coding regions of ~70%. This observed pattern with insertions in genes is believed to decrease the risk of an insertion leading to a disruptive gene regulation promoting transformation.

Genes targeted by the vector were analysed in more detail. 1448 genes (out of 21374 in total) were identified as targets for Lenti-D LVV integration. Out of these, 151 targets were annotated as transcription regulators. Integration hotspots were investigated by determining the occurrence of common integration sites (CIS). CIS are regions in which multiple vector integration occurs and are thought to be an indicator for possible clonal selection. Approximately 19 % av all IS were found to be CIS and the applicant has provided information on the genes targeted**.** To conclude, the ISA results suggest an insertion preference consistent with other LVV described in the literature. There were no signs of clonal selection and the overall potential for genotoxicity is considered sufficiently low. It should however be noted that predominant clones have been reported in two human patients receiving Lenti-D.

Carcinogenicity

No traditional life-time rodent carcinogenicity studies were performed, which is acceptable. However, the applicant points out that no signs of tumorigenicity was observed in any of the non-clinical studies. This is not considered predictive for humans.

2.3.4. Environmental risk assessment

Skysona contains a Lenti-D lentiviral vector that is replication defective, self-inactivating. The lentiviral vector is used for the ex-vivo transduction (genetic modification) of autologous human CD34+ cells. The transduced cells express ALDP.

Lenti-D Drug Product is a genetically modified organism (GMO) product and is a such assessed through a GMO environmental risk procedure approach. Skysona is not intended for deliberate release into the environment and cannot survive if accidentally released into the environment. The predicted behaviour of Skysona and the environmental impact of the product is not considered to be different from the non-modified CALD CD34+ HSCs. As such, shedding of modified cells via e.g. the blood is unlikely to be an issue. The risk to the environment in therefore considered negligible.

Another shedding possibility is if there are residual lentiviral vectors (LVV) in the product, but the quality strategy by the applicant supports the notion that the presence of such LVVs is negligible.

Overall, the risk to the environment, human or animal health of shedding of viral particles is negligible.

In terms of risks for non-patients, this mainly leaves accidental transferal to non-target persons (e.g. via accidental transfer healthcare professionals or patients, via transfusion of blood or transplantation of cells, tissues or organs from a donor that has been treated with Skysona). A risk for non-human organisms is considered negligible. Among those 'accidental' transmission to non-target person scenarios, Nominally, Skysona (modified CALD CD34+ HSCs) would have no different human health effects than the recipient (non-transduced) CALD CD34+ HSCs. Yet associated with such an accidental event (especially in immunodeficient persons) is that any residual LVVs may initiate insertional mutagenesis that may lead to oncogenesis (considered a theoretical possibility for Skysona patients).

The replication-defective nature of the LVV prevents the possibility of spontaneous mobilisation of the integrated vector from the transduced cells unless helper functions are provided in the transduced cells by super-infection with wild-type virus (e.g. HIV or HTLV) in an infected host. The self-inactivating feature (SIN) of the vector LTR inhibits vector mobilisation even in the case of superinfection of the transduced cell by a wildtype virus. There are also risk minimisation procedures such as viral screening in putative patients/donors, making the overall risk for this hazard negligible in non-target persons. Therefore, Skysona is predicted to have overall negligible environmental impact.

Adequate measures are implemented in the product information to prevent risks of accidental transfer during administration to health care professionals involved in the handling/administering the product. Adequate measures are also put in place for storage, transportation and waste treatment. Patients are also asked to refrain from donating blood/cells/tissues/organs after being administered Skysona.

2.3.5. Discussion on non-clinical aspects

Pharmacology

There was a direct relationship between MOI and VCN values and %ALDP+ cells after Lenti-D LVV transduction of human ALDP-deficient FBs with MOI ranging from 0.3 – 5.5 (study NC-12-003). I is noted the % ALDP+ cells in *in vitro* cultured ALD donor FBs appear to be slowly diminishing over the limited time studied (up to three weeks). A reduction in % ALDP+ cells was also observed in clinical trials at early time points after treatment, but the level was stabilised at 6 months after treatment and remained stable at 24 moths and at latest studied time point, 60 months after treatment.

A trend for lower colony counts in transduced, as compared to mock-transduced cells suggested a possible effect by the Lenti-D LVV transduction on cellular growth and proliferation. However, the applicant put forward that in an *in vitro* immortalisation (IVIM) study, transduction of mouse BMCs with Lenti-D LVV at MOI 120 resulted in a VCN value of 4.78 c/dg and was not associated with cytotoxic effects. Nevertheless, in several of the other performed studies the colony counts appeared lower at higher MOIs. The applicant has provided clarification on this issue and it is likely that the initial reduction of engrafting cells is not a result of cytotxicity due to high MOIs. It is also noted that the MOIs used in the *in vitro* nonclinical studies were substantially higher than are used in the manufacture of Lenti-D Drug Product, which supports the safety of the clinical product.

In the transplanted NSGS mice, it was not possible for the applicant to demonstrate specific staining of hALDP+ cells within the brain, although migration to the brain of human CD45+ cells with microglial phenotype was demonstrated. The applicant provides two potential explanations for this. First, a substantial non-specific staining was observed in the mouse brains. Furthermore, the absence of specific hALDP staining in mouse brains is not inconsistent with the relative absence of hALDP+ cells in normal human brain. These potential explanations could be acceptable. Considering the relatively modest increase in fluorescens signal (33%) after ALDP -staining of hCD45+/hALDP+ BM cells in the NSGS mice transplanted with Lenti-D LVV compared to mock-transduced human healthy donor (ALDP wild type) mPB-derived CD34+ HSCs, ALDP-brightly stained cells above background was perhaps not to be expected. The "background" normal tissue expression of ALDP in wildtype mouse and human tissues has been demonstrated to be comparable (Fouquet et al. 1997).

In summary, BM and brain engraftment by Lenti-D LVV (and mock-) transduced human mPB CD34+ cells have been demonstrated in the immune compromised NSGS mouse model. It is unclear why no ALDP+ cells could be identified in the brain after infusion with the Lenti-D LVV transduced cells, although >24% of the CD34+ cells were shown to be ALDP+ before infusion and thus a fifth of the counted CD34+ cells theoretically could be ALDP+. The clinical situation can, however, not be expected to be fully represented due to the translational gap between this mouse model and patients. Thus, the

study can be considered acceptable as a nonclinical proof of principle, that Lenti-D LVV transduced human mPB CD34+ cells can engraft the BM and also migrate to the brain and there show microglial phenotypic markers.

Demonstration of Lenti-D LVV-mediated correction of VLCFA metabolism *in vitro,* using ALD FBs and AMN CD34+ HSCs, was suggested by the applicant to constitute a preclinical PoC since there are no animal models of CALD that recapitulate the human disease and could be used for demonstration of improvements in cerebral inflammation and demyelination after administration of Lenti-D-transduced CD34+ HSCs. However, in the assessment this is not considered as a regular PoC. Nevertheless, taken together with the indicated brain engraftment, these both observations provide acceptable preclinical support for the treatment concept. The translational gap between the xenotransplantation model with transduced human cells infused to immunocompromised mice as compared to patients, precludes addressing more detailed clinical questions since that would require, among other things, an optimal environment for the differentiation and migration of human HSCs, which so far has not been possible to establish in an animal model.

No secondary pharmacological studies have been carried out. The autologous components of the Skysona product is not expected to exert any off-target effects within the physiological range. However, off-target effects cannot be excluded at overexpressed levels or possibly at non physiological distribution. The applicants definition of secondary pharmacodynamics appears to parallel the primary pharmacodynamic effect. However, there were no indication of any safety related findings potentially related to secondary pharmacology in the toxicology part. Considering the class and nature of this autologous cell drug product, the absence of dedicated secondary pharmacodynamic studies is accepted.

No dedicated safety pharmacology studies have been conducted. In addition, to the absence of overt safety pharmacology findings in the animal studies, there is no indications of any findings potentially related to safety pharmacology in the clinical studies, though it should be considered the patients undergo a conditioning treatment possibly masking potential safety related findings. Considering the class and nature of this autologous cell drug product, the absence of dedicated safety pharmacology studies is accepted.

Toxicology

The applicant considers the finding of a HMGA2 insertion in one mouse to be of little or no relevance to the oncogenic potential of Lenti-D and has provided a discussion on IS in the HMGA2 gene. The relevance of the mouse observation is considered unknown by the applicant since the effect on oncogenicity is dependent on the exact position of the insertion. This is acknowledged. It should however be noted that two human subjects receiving Lenti-D have emerged with predominant clones, so far without clinical consequences. In both cases the clone appears to be of myeloid lineage and both subjects had an IS in the MECOM gene. This is further discussed in the clinical part.

Although the samples in the ISA were within the range VCN used in clinical trials (0,5-3,1), none of the samples had VCN values equal to or above the highest VCN used clinically (3,1) This is not optimal since increasing VCN levels are expected to lead to an increase in the number of insertions. Also, the cells used in the ISA appear to be from a single donor and transduced with a single Lot (batch) of Lenti-D LVV. However, these shortcomings are not considered enough for demanding additional analysis and could be considered covered by the clinical monitoring, were subjects in the clinical studies are monitored by ISA every 6 months up to 5 years after treatment and patients are to be monitored annually for leukemia/lymphoma for 15 years post treatment.

Environmental risk assessment

Skysona is not intended for deliberate release into the environment and cannot survive if accidentally released into the environment. As such, shedding of modified cells via e.g. the blood is unlikely to be an issue. Another shedding possibility is if there are residual lentiviral vectors (LVV) in the product, but the quality strategy by the applicant supports the notion that the presence of such LVVs is negligible. In terms of risks, this mainly leaves accidental transferal to non-target persons (e.g. via accidental transfer healthcare professionals, via transfusion of blood or transplantation of cells, tissues or organs from a donor that has been treated with eli-cel). Considering the risk management scenarios, the vector itself (e.g. SIN properties) and the required conditions in the nonpatient for a possible effect (e.g. immunodeficient,), eli-cel is predicted to have overall negligible environmental impact. It can be noted since the modified CALD cells cannot survive in the environment outside the human body, no methods of detection have been developed for the purpose of environmental monitoring. But eli-cel could be still detected by adequate polymerase chain reaction (PCR) methods that have been designed to amplify a portion of the integrated cDNA (psi-gag) or by sequencing cellular gDNA (see Quality aspects) – e.g. in the case of accidental transmission to third parties.

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

2.3.6. Conclusion on non-clinical aspects

As Skysona is an autologous cell-based gene therapy product, conventional non-clinical studies might not always be relevant. Overall, the pharmacological studies provide evidence that support efficacy in humans. The safety data from immune-deficient mice are considered less relevant in predicting the human situation. When it comes to cell therapies, it is well established that animal models are poor predictors of safety in humans. Therefore, the persistence and safety of Skysona can eventually only be determined by clinical use. When it comes to genetic safety, the integration pattern of skysona is considered to, overall, be consistent with previously described LVV integration profiles with preferred integration in gene-coding regions across the whole genome. It is however noted that two human subjects receiving Skysona have emerged with predominant clones, so far without clinical consequences. In both cases the clone appears to be of myeloid lineage and both subjects had an IS in the MECOM gene. This is further discussed in the clinical part. Skysona is predicted to have overall negligible environmental impact and adequate precautions are included in the product information for handling, disposal or accidental exposure.

From a non-clinical perspective, the MAA for Skysona can be approvable, if the B/R is considered positive for quality and clinical side.

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

2.4. Clinical aspects

2.4.1. 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	Objective of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Elivaldogene autotemcel							
ALD-102	Efficacy and safety	Open-label, multi-centre, single arm, externally controlled	eli-cel; ≥ 5.0×10^6 CD34+ cells/kg; Intravenous infusion	32 treated	Patients ≤17 years with CALD	Single dose	Ongoing; Interim (full)
ALD-104	Efficacy and safety	Open-label, multi-centre, single arm, uncontrolled	eli-cel; ≥ 5.0×10^6 CD34+ cells/kg; Intravenous infusion	13 treated	Patients ≤17 years with CALD	Single dose	Ongoing; Interim (safety focus)
LTF-304	Long-term follow-up from parent studies; safety and efficacy	Long-term follow-up, single arm, uncontrolled	N/A (dosed in prior studies)	21 enrolled	Patients ≤17 years with CALD treated with eli-cel in parent study	N/A	Ongoing; Interim (full)
Allogeneic HSCT and/or Untreated Historical Controls							
ALD-101	The natural history of disease in untreated subjects with CALD; Efficacy and safety of allo-HSCT in subjects with CALD	Retrospective, non-interventional, data collection study	N/A	137 (72 untreated; 65 allo-HSCT treated)	Patients ≥ 3 and ≤ 15 years with CALD	N/A	Complete; Final (full)
ALD-103	Safety and efficacy of allo-HSCT in subjects with CALD	Retrospective and prospective, data collection study	N/A	59 allo-HSCT treated	Patients ≤ 17 years with CALD	N/A	Complete; Final (full)

2.4.2. Pharmacokinetics

Elivaldogene autotemcel (eli-cel) is a gene therapy medicinal product intended for the treatment of cerebral adrenoleukodystrophy (CALD). The active substance is an autologous CD34+ cell-enriched

population that contains haematopoietic stem cells transduced with a lentiviral vector (LVV) encoding *ABCD1* cDNA for human adrenoleukodystrophy protein (ALDP).

Skysona is presented as a dispersion for infusion; cells are suspended in Cryostore CS5 cryopreservation solution containing 5% dimethyl sulfoxide (DMSO). Eli-cel is supplied in 20 ml fluorinated ethylene propylene (FEP) bags and is administered as a single dose by intravenous infusion. Each lot contains between 40 x 10^6 and 1200 x 10^6 cells.

The pharmacokinetics of the vector – in terms of vector copy number (VCN) - and transgene product were studied in studies ALD-102, 104 and their follow-up LTF-304. A number of analytical methods were used for this purpose. The VCN and ALDP data is presented in the pharmacodynamics section below.

Each analytical method was available in two versions, unless noted otherwise. The following methods were used to monitor subjects after treatment with eli-cel for the presence of transgene sequences, transgene expression, and replication competent vector, as well as to identify the genomic integration sites present in clinical samples:

- A Cell-sorting Method for Separation of Peripheral Blood Cells into Different Lineages Using Magnetic Beads
- Quantitative Polymerase Chain Reaction Methods to Detect Lenti-D LVV Proviral DNA Sequences in Human Genomic DNA for Quantification of Vector Copy Number within Cell Samples
- Flow Cytometry Method for Detection of ALDP+ Cells in Peripheral Blood
- Methods for Screening Blood Samples from Subjects Receiving Eli-cel for Replication-competent Lentivirus
    - An Enzyme-linked Immunosorbent Assay Method for the Detection of HIV-1 Protein in Human Serum as a Screening Assay for RCL
    - A qPCR Method for the Detection of Sequences in DNA from Human Blood as a Screening Assay for RCL
- A Method for Confirming the Presence of RCL
- Integration Site Analysis
- Very long chain fatty acid method

No assay was available to detect anti vector or anti ALDP antibodies.

Cell-sorting method

This method aimed at separating blood cells into T cells (CD3+), monocytes (CD14+), neutrophils (CD15+) and B-cells (CD19+) for use in downstream applications such as VCN determination, ALDP+ determination and integration site analysis (ISA). The procedure uses commercially available magnetic microbead-conjugated monoclonal antibodies, which recognize and bind to specific molecules on the surface of the cells of interest. When the sample is subjected to a magnetic field, the cells of interest, bound to the magnetic beads, can be separated using positive selection, where they remain in suspension to collect the cell populations of interest. All cell subtypes were separated with average purities of at least 87% at CCHMC, while purity was slightly lower at PPD (62-91% depending on cell type).

HIV qPCR

This qPCR assay was used to quantify vector copy numbers in transduced cells. Because the vector copy number (VCN) is determined on a heterogeneous population of cells, VCN is a composite value, dependent both on percent of cells transduced (percent of cells LVV-positive, %LVV+) as well as number of copies per transduced cell. VCN measured in drug product, which is comprised largely of CD34+ cells, is referred to as DP VCN; VCN measured in peripheral blood leukocytes is referred to as PB VCN; and VCN measured in CD14+ monocytes is referred to as CD14+ VCN.

Two closely related qPCR methods for the detection of HIV-1 sequences in human genomic DNA (gDNA) and quantification of VCN values within cell samples were validated using the K3 cell line as a control. It is a diploid human-derived cell line, so that the target gene and the internal reference genes exist in a 1:1 ratio. The main difference between the method at CCHMC and PPD was the reference gene. A plasmid containing psi-gag and the reference gene was used for quantitation.

Flow Cytometry Method for Detection of ALDP+ Cells in Peripheral Blood

Relative to CALD HSCs with a mutated *ABCD1* gene, the transduced CALD HSCs and their progeny will over-express ALDP when analysed by flow cytometry, allowing for a calculation of the relative numbers of ALDP+ cells, defined as the percentage of cells that stained above background (i.e., above endogenous ALDP levels in non-transduced cells) in drug product, peripheral blood or CD14+ cells.

For intracellular staining of ALDP, fresh (up to 96h after collection) cells are permeabilised with a detergent or alcohol to allow antibodies against intracellular antigens to enter the cell, followed by indirect staining, where ALDP is reacted with an unlabelled primary antibody, followed by a fluorochrome-conjugated secondary antibody specific for the primary antibody.

Both CCHMC and PPD methods used peripheral blood monocytes, which serve as a surrogate marker for HSCs and their progeny. Both methods were qualified using whole blood from healthy donors as negative control and CD34+ cells transduced with Lenti-D LVV as positive control.

At CCHMC, the assay can consistently detect down to 1.5 % ALDP+ cells with a precision of 72-106% and can reliably quantify ~ 4% ALDP positive cells – considering occasional outliers.

At PPD, the LLOQ was determined to be 3% ALDP+ cells. Additionally, simultaneous staining with an Alexa Fluor 647-conjugated mouse anti-human CD14 IgG2a antibody CD14 enabled gating of CD14+ cells without using magnetic bead separation first. Samples from healthy donors give a baseline of < 1% ALDP+ cells. Similarly, CALD patients prior to eli-cel administration also have non detectable or < 1% ALDP+ cells.

Screening and confirmation of replication competent lentivirus (RCL)

Two methods for RCL were used to screen blood samples from subjects who had received eli-cel: an enzyme-linked immunosorbent assay for HIV-1 and a qPCR assay.

An enzyme-linked immunosorbent assay (ELISA) method utilising a commercially available kit for the detection of HIV-1 protein in human plasma or serum was qualified. The HIV-1 method was found to lack sensitivity and result in repeated false positive results due to interferences. The qPCR method was therefore used instead. In study ALD-102, all samples from subjects' certain subjects, and samples at month 3, 6 and 12 of other subjects were analysed only with the ELISA assay. All results from these subjects were negative.

A qPCR assay for the detection of in DNA from human blood was adopted as a method to screen for RCL. The method was validated. All samples tested with this method were RCL negative. Two samples in study ALD-104 had detectable levels, however below the limit of quantification (<10 copies/0.2 µg DNA).

A bioassay was intended for the confirmation of RCL. Since all samples were negative at screening, this assay was not used for samples included in this MAA.

Integration site analysis (ISA)

ISA determines patterns of integration of Lenti-D LVV in gDNA from peripheral blood of subjects treated with eli-cel and identifies the relative contribution of individual IS over time to define an increase or decrease in the contribution of individual IS. ISA was based on two published methods (Schmidt et al. 2001, 2007) and performed as a qualified and validated method at GeneWerk (Heidelberg, Germany).

From 2014 up to 31 May 2019, ISA was done by standard linear amplification PCR (LAM-PCR) and non-restricted LAM-PCR (nrLAM-PCR); together referred to as [nr]LAM-PCR. [nr]LAMPCR was the assay of choice in the early stages of our clinical studies because of its sensitivity in detecting low-abundance integration sites (IS) in samples with a low VCN. However, the use of restriction enzymes in [nr]LAM-PCR can introduce amplification biases that can result in inaccurate estimations of IS frequencies (abundance).

For [nr]LAM-PCR, peripheral blood gDNA was subjected to PCR using biotinylated primers that hybridize to the 3'-region of the LTR of the vector. This was followed by magnetic capture of the biotinylated PCR-products, double-strand DNA synthesis, restriction digestion as relevant, and addition of linker adaptors containing a molecular barcode. The digested fragments were then subjected to exponential PCR using nested vector- and adaptor-specific biotinylated primers. The PCR products were then subjected to deep sequencing followed by bioinformatics analysis to identity unique, mappable integration sites and to determine their relative frequency amongst all IS. The data was then analysed to determine whether predominant clones were present, with a semi-quantitative readout.

As of 01 June 2019, samples were analysed by shearing extension primer tag selection ligation-mediated polymerase chain reaction (S-EPTS/LM-PCR). S-EPTS/LM-PCR substantially minimizes the risk that individual IS may be masked, as may happen with [nr]LAM-PCR if the next restriction site is very close in distance to the vector integrant and thus not able to be mapped to the host genome (S-EPTS/LM-PCR Validation Report Summary, 24 June 2020).

For S-EPTS/LM-PCR, sheared gDNA was purified, and primer extension was performed using an LTR specific biotinylated primer. The extension product was again purified, followed by magnetic capture of the biotinylated DNA. The captured DNA was then ligated to linker cassettes, including a molecular barcode and the ligation product amplified in the first exponential PCR, using biotinylated vector- and linker-cassette-specific primers. The biotinylated PCR-product was magnetically captured and pooled. The eluted product was used as template for a second exponential PCR and the product was purified and subjected to deep sequencing by MiSeq technology (Illumina) followed by bioinformatics analysis to identify unique, mappable integration sites. Positive controls in the validation were genomic DNA from clone L2E9 bearing an integration site in RERE gene and clone L3D7 showing an integration site in PTPN13 gene as well as genomic DNA derived from a polyclonal lentiviral vector-infected bulk population. The method was validated for specificity, linearity, within and between run accuracy and precision with acceptance criteria being CV ≤20%. The limit of detection was at a clonal distribution 0.05%, which corresponds to the detection of 34.09 RERE integration site copies within a pool of 68,181.8 integration sites.

Regardless of screening method used, a verification of Relative IS-Frequency of any IS identified as of interest, according to protocol-defined criteria (e.g., detected at a frequency of >30% at any timepoint, or persistently >10% in Study Protocol ALD-104 v4), is analysed further by qPCR using customised IS-specific primers. Customised primers were designed for each IS of interest, and their relative contribution to the overall vector copies in the sample, was determined by comparison with the

total number of vector sequences as determined by using primers specific for the psi-gag region of the LVV.

Very long chain fatty acid (VLCFA) method

Fasting serum VLCFA levels were quantified in subjects at screening and at pre-defined intervals post eli-cel infusion, to monitor for changes. The validated method used capillary gas chromatography-electron-capture negative-ion mass spectrometry of fatty acids, based the published method (Lagerstedt et al. 2001). Levels of C26:0, C26:1, C24:0, C22:0 and C22:1 were determined, as well as the ratios of C24:C22 and C26:22. No validation report was provided.

Conditioning, lymphodepletion and PK monitoring

Myeloablation of the subjects before drug product infusion is required to deplete endogenous HSCs, thus allowing repopulation of the subject with HSCs containing the transgene without dilution due to the presence of residual unablated cells. Current literature indicates that myeloablation is required for effective reconstitution with transplanted HSCs; reduced intensity conditioning generally has shown lower rates of donor cell reconstitution. The conditioning included lymphodepletion before Eli-Cel infusion because CALD is associated with lymphocytic infiltration. Two different lymphodepletion regimens were used; busulfan and cyclophosphamide in study ALD-102 or busulfan and fludarabine in study ALD-104, which reflected clinical practice and the introduction of a less toxic conditioning regimen. Regardless of conditioning regimen, sufficient lymphodepletion was achieved to allow eli-cel treatment.

ALD-102

During conditioning, the target daily busulfan AUC range was 4250 to 5250 µM*min and in 26 out of 31 subjects busulfan AUC was within target range, as estimated by the cumulative busulfan exposure, based on measured concentration from the first dose of subsequent dose if required. One subject was excluded due to data entry error and the remaining 5 subjects had daily busulfan AUC's below the target range. In order to clarify whether the conditioning was insufficient, the ratio of PB VCN at Month 6/DP VCN was plotted against estimated average busulfan AUC, denoting no apparent correlation.

ALD-104

During conditioning, the target busulfan AUC range was 5340 to 5964 µM*min/L/day and in 6 out of 13 subjects busulfan AUC was within target range. The remaining 7 subjects had daily busulfan AUC's below the target range. In 6 of these subjects, busulfan doses were gradually increased following low initial AUC values and subsequently AUC fell within target range. One subject with initial busulfan AUC above target range fell below after dose adjustment. All subjects experienced effective myeloablation and afterwards production of gene modified cells, also the seven patients who had busulfan daily average AUC below target interval.

Special populations

No data is available in patients with impaired renal or hepatic function. All patients were male, as the disease affects predominantly males.

Eli-cel is dosed on the basis of the number of CD34+ cells per kg body weight, a practice that is followed for bone marrow transplantation, including treatment using allo-HSCT for children who have CALD. No correlation was observed between body weight and PB VCN or PB %ALDP+ cells. No dose adjustments are required for overweight subjects.

Given the target population, no elderly were included in clinical trials with eli-cel.

	Age 65-74 (Older subjects number)	Age 75-84 (Older subjects number)	Age 85+ (Older subjects number)
PK Trials	0	0	0

Interactions

No formal drug interaction studies have been performed. Eli-cel is not expected to interact with the hepatic cytochrome P 450 family of enzymes or drug transporters.

Patients should not take anti-retroviral medicinal products from at least one month prior to mobilisation until apheresis is completed.

The safety of immunisation with attenuated viral vaccines during or following eli-cel treatment has not been studied. In clinical studies, patients received attenuated vaccines following eli-cel treatment.

Exposure relevant for safety evaluation

In the pivotal study ALD-102, median PB VCN was 0.751 c/dg in PBLs, and 0.932 c/dg in CD14+ cells after 1 month. After 24 months, median PB VCN was 0.436 c/dg in PBLs, and 0.485 c/dg in CD14+ cells. The median percentage ALDP+ cells in peripheral blood was 18.75% and 8.35% after 1 and 24 months, respectively. The median percentage ALDP+ cells in CD14+ cells from peripheral blood was 29.50% and 17.65% after 1 and 24 months, respectively.

2.4.3. Pharmacokinetics/Pharmacodynamics

Eli-cel adds functional copies of the *ABCD1* cDNA into patients' HSCs through transduction of autologous CD34+ cells with Lenti-D LVV. After eli-cel infusion, transduced CD34+ HSCs engraft in the bone marrow, and subsequently differentiate into various cell types, including monocytes that migrate to the brain where they further differentiate into long-lived macrophages and cerebral microglia that can produce functional ALDP and replace deficient microglial cells. The functional ALDP can then enable the local degradation of VLCFAs in the brain, which in turn can stabilize the disease by preventing further inflammation and demyelination. Following successful engraftment with genetically modified cells, the expression of functional ALDP is expected to be lifelong.

No dedicated pharmacodynamics study was performed, instead various pharmacokinetic/pharmacodynamic parameters were measured in all clinical studies of eli-cel (ALD-102, ALD-104, LTF-304) and serum VLCFAs was also measured in the historical control study ALD-103 with allo-HSCT as treatment. In the drug product, the median DP VCN was lower in study ALD-102 (1.20, span 0.5-2.7) than in study ALD-104 (1.70, span 1.2-3.1) and the median DP%LVV+ cells were 45% (span 19-67%) in study ALD-102 and 63.5% (span 41-84%) in study ALD-104. The median number of vector copies per transduced cell (DP VCN/DP%LVV+Cells) was 2.60 (1.8-4.5) in study ALD-102 and similar in study ALD-104 (2.65 (2.2-3.7).

Table 2 Summary PK/PD for study ALD-102 and LTF-304 vs ALD-103

Parameter (Median (min, max))	**Baseline**	**Month 1**	**Month 6**	**Month 12**	**Month 24**	**Month 48**
PBL VCN (c/dg)	N/A	N=31	N=31	N=31	N=20 0.44 (0.05, 1.67)	N=13 0.47 (0.06, 1.49)

		0.75 (0.10, 1.88)	0.52 (0.07, 2.23)	0.41 (0.07, 3.24)		
CD14+ VCN (c/dg)	N/A	N=26 0.93 (0.11, 1.82)	N=29 0.61 (0.07, 3.96)	N=30 0.48 (0.08, 4.00)	N=20 0.49 (0.06, 1.86)	N=14 0.49 (0.06, 1.69)
PBL %ALDP+ cells	N=27 0.87 (0.10, 2.50)	N=30 18.75 (3.90, 34.97)	N=31 11.83 (1.50, 46.77)	N=30 13.80 (1.00, 37.40)	N=20 8.35 (3.80, 26.60)	N=13 7.95 (3.14, 40.02)
CD14+ %ALDP+ cells	N=25 1.00 (0.30, 4.20)	N=25 29.50 (8.20, 49.65)	N=27 22.20 (3.20, 71.40)	N=29 16.50 (5.70, 54.45)	N=20 17.65 (6.30, 44.60)	N=13 12.48 (4.39, 47.08)
VLCFA: C26:0 LysoPC (% change from baseline)	No data	No data	N=32 5.2 (-64.8, 102.5)	N=31 -6.4 (-65.5, 56.9)	N=20 -20.2 (-58.5, 94.4)	No data
VLCFA: C26:0/C22:0 ratio (% change from baseline)	No data	No data	N=32 -10.6 (-44.4, 40.0)	N=31 -14.3 (-60.0, 60.0)	N=20 -15.5 (-50.0, 50.0)	No data
Study ALD-103 (TPES)						
VLCFA: C26:0 LysoPC (% change from baseline)	No data	No data	N=8 -46.0 (-77.1, 46.1)	N=7 -62.6 (-81.9, -38.8)	N=5 -61.2 (-74.9, 5.2)	No data
VLCFA: C26:0/C22:0 ratio (% change from baseline)	No data	No data	N=8 -39.6 (-50.0, 16.7)	N=7 -37.5 (-62.5, -16.7)	N=5 -28.6 (-58.3, 0.0)	No data

PBL= peripheral blood leukocytes, VCN= vector copy number (c/dg), CD14+ = peripheral blood monocytes, % ALDP+ cells= percentage ALD protein-positive cells, VLCFA= very long chain fatty acids, LysoPC=lysophosphatidylcholine, TPES=Strictly ALD-102 eligible population

Table 3 VLCFA in Fasting Plasma in Normal Males, ALD Males, and Obligate Heterozygote Carriers

VLCFA	Statistic	Normal Adult Males	Obligate Female Carriers	X-ALD Hemizygote Males
C26:0 (µg/mL)	Mean (SD)	0.23 (0.09)	0.68 (0.29)	1.30 (0.45)
C24:0/C22:0	Mean (SD)	0.84 (0.10)	1.30 (0.19)	1.71 (0.23)
C26:0/C22:0	Mean (SD)	0.01 (0.004)	0.04 (0.02)	0.07 (0.03)
C26:0 LysoPC (nmol/L)	Min, Max	10.6, 99.1	22.7, 698.2	157.5, 1266.6

Source: Kennedy Krieger Institute
Abbrev.: ALD, adrenoleukodystrophy; C22:0, docosanoic fatty acid; C24:0, tetracosanoic fatty acid; C26:0, hexacosanoic fatty acid; LysoPC, C26:0 lysophosphatidylcholine; SD, standard deviation; VLCFA, very long-chain fatty acids.

Vector copy numbers per cell in peripheral blood was measured both in leukocytes and CD14+ monocytes (considered the best proxy for VCN levels in microglia cells in CNS) and was gradually declining from initial values of 0.75 to 0.9 over the initial 12 months after treatment, after which it stabilised at about 0.5 in both cell types through month 60. In the study ALD-102, by-subject VCN in PBLs and CD14+ over time demonstrated intra-individual and interindividual variation, which was attributable to high values observed in two subjects.

Proportion of ALDP positive cells (%ALDP+) was substantially increased through month 60 compared to low baseline values, but gradually declined throughout the measurement period from its highest values at month 1 post treatment for leukocytes, whereas in monocytes it appeared to stabilise from month 12.

The serum very long chain fatty acids (VLCFAs) concentration was gradually decreased over the 24 months in study ALD-102 reaching the largest decrease of -20%, as measured by C26:0 LysoPC. In study ALD-104, -22% in C26:0 LysoPC was measured in the 8 patients with month 12 data available. However, this decrease was considerably lower than the corresponding results of -61% at month 24 achieved after allo-HSCT in study ALD-103.

When analysing the potential relationship between vector copy numbers in peripheral blood with effects on VLCFA lowering, there was no correlation. Neither was any correlation seen between cell dose and time to neutrophil or platelet engraftment. In addition, no correlation was seen between clinical outcome (as measured by MFD-free survival) and vector copy number in the drug product. However, it was noted that for 2 of 3 patients not reaching the primary endpoint MFD-free survival at month 24, the drug product vector copy number was among the lowest observed at 0.5 c/dg.

The applicant states that the presence of the transgene is expected to be lifelong after eli-cel infusion. Detectable PB VCN up to 60 months after eli-cel infusion, has been demonstrated, but the IQR and mean %ALDP+ cells in PB seem to decline over time. The long-term data are, however, limited by few subjects.

2.4.4. Discussion on clinical pharmacology

Pharmacokinetics

Conventional pharmacokinetic characterisation of the product is not possible and not expected for GTMPs (Gene Therapy Medicinal Products). As stated in the GTMP guideline (EMA/CAT/80183/2014), PK studies should focus on the distribution, persistence, clearance and mobilisation of the GTMP and address the risk of germline transmission. These are described in the pharmacodynamics section and consequently in section 5.1 of the SmPC instead of section 5.2, which is acceptable.

Analytical methods

Cell sorting

The cell sorting method – at both locations – is considered fit for its purpose. Differences of purity of the different cell types are seen for the two methods. There is no consequence of the purity on subsequent analyses as there is no difference in the transduction of the different nucleated leukocytes.

Vector copy number

The qPCR method at PPD is considered adequate for the measurement of VCN as sufficient sensitivity, linearity, specificity, intra- and inter-day precision, accuracy and long-term stability were demonstrated.

The qPCR method at CCHMC and PPD use a different gene for normalisation. As the assays are based on genomic DNA the impact of this difference on assay outcome is likely to be minimal. All results were reported as copies/cell in the clinical studies.

ALDP+ cells

The CCHMC method has shown sufficient specificity, sensitivity, precision, consistency and reproducibility and can be considered qualified for the intended use. LOD was 1.5% ALDP+ cells, while LOQ was ca 4% using the CCHMC assay.

Clarification on the PPD assay was provided, LLOQ was 3% ALDP + cells. Untransduced cells (healthy and CALD patients´) are below LLOQ. ALDP+ cells referred to low expressing ALPD cells, while ALDP++ were high expressing cells. The gating strategy had to be revised after observation of different staining profiles in study samples compared to the spiked samples used in the method qualification. It is understood that the revised gating strategy was applied to all clinical study samples. The assay is considered fit for the intended purpose.

RCL

The ELISA assay was not fully validated according to GL, in particular the criteria for setting the cutpoint were not based on 50 different donors. However, issues were identified with the assay, leading to the use of an alternative method. Clarification was provided on which data from study ALD-102 was is solely based on the ELISA assay. Since all results were negative, the issue is not pursued.

The qPCR method was confirmed to be identical to that described by Sastry et al. The method has acceptable reproducibility and sensitivity.

Linearity, intra and inter-day precision and accuracy of the qPCR method are not specifically addressed in the method qualification. Since the method is sensitive and reproducible, it may nevertheless be considered appropriate for the use as a screening assay for RCL.

The confirmatory co-culture assay is reproducible with positive controls of a well-described virus, the assay is deemed fit for its purpose. Of note, the assay was not used as all samples were negative at screening.

Integration site analysis

The [nr]LAM-PCR method was only described in the summary of clinical biopharmaceutics and was not validated nor GLP compliant, however, according to the applicant the methods were "fit-for-purpose" and conducted with a focus on high quality and reliability. Sufficient information is provided to give confidence in the assay and the outcome of the analysis. Therefore, and also given the specificities and complexities of the assay and subsequent analysis, the absence of validation and GLP compliance is accepted.

The S-EPTS/LM-PCR was adequately validated; it is however not CE marked.

Very long chain fatty acid (VLCFA) method

No details were provided on the validation of the VLCFA method. As this is a standard diagnosis procedure, this is acceptable, and the method is considered fit for its purpose.

Conditioning, lymphodepletion and PK monitoring

The conditioning included lymphodepletion before eli-Cel infusion because CALD is associated with lymphocytic infiltration. Two different lymphodepletion regimens were used; busulfan and cyclophosphamide in study ALD-102 or busulfan and fludarabine in study ALD-104. The applicant clarified that this reflected the evolving clinical practice, with the introduction of a less toxic conditioning regimen. Regardless of conditioning regimen, sufficient lymphodepletion was achieved to allow eli-cel treatment.

The applicant provided the missing plots to support the adequacy of the conditioning. In study ALD-102, the ratio of PB VCN at Month 6/DP VCN was plotted against estimated average busulfan AUC. No correlation was found.

In study ALD-104, the applicant investigated whether the conditioning was sufficient by monitoring of white blood cell counts. It indicated that all subjects in Study ALD-104 were effectively myeloablated, including subjects with busulfan AUC below target range**.**

Special populations

Limited data is available on special populations. Organ function, gender, age or race are not expected to have an impact on the PK/PD.

The patients in the development programme constitute a homogenous population and the small numbers do not allow to identify intrinsic factors related to safety or efficacy. The pharmacokinetics in elderly and patients with impaired renal- or hepatic function has not been investigated, which is acceptable. Due to the nature of the disease, age is not of relevance. Likewise, as the product does not exert conventional PK like small-molecules, impaired renal- or hepatic function is not expected to impact the exposure-safety or exposure-efficacy relationship.

Bodyweight and the administered dose did not have a significant effect on PD outcomes, thus capping of the dose is not necessary in adolescents and overweight paediatric patients.

Interactions

The lack of interaction study is acceptable. The interaction potential is expected to be low given the nature of the medicinal product. Similarly, the lack of data in patients with renal or hepatic impairment is acceptable in view of the nature of the medicinal product.

The contraindication for anti-retroviral products is related to the manufacturing of the product and is acceptable.

Pharmacokinetics/pharmacodynamics data

The PD of Eli-cel has been established in accordance with the requirements of the Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008 Rev. 1). Stem cell engraftment has been confirmed by investigating VCN in PBLs and CD14+ cells over time and production of pharmacologically active levels of target ALDP+ PBLs and CD14+ cells.

With regards to drug product (DP) attributes, the median DP VCN was lower in study ALD-102 (1.20, span 0.5-2.7) than in study ALD-104 (1.70, span 1.2-3.1). This is at least partly related to the DP VCN specification lower limit being increased from 0.5 to 0.7 c/dg on 19 Jan 2017, when study ALD-102 was ongoing and study ALD-104 had not started yet. The difference between the studies in DP VCN is also reflected in the median DP%LVV+ cells, being 45% (span 19-67%) in study ALD-102 and 63.5% (span 41-84%) in study ALD-104.

In study ALD-102, the peripheral blood (PB) leukocytes VCN and CD14+ VCN over time was higher during the initial 6-12 months after eli-cel gene therapy but stabilised at a lower level thereafter. By month 1 after receiving eli-cel, median (min, max) VCN was 0.751 (0.10, 1.88) c/dg for PBLs and 0.932 (0.11, 1.82) c/dg for CD14+ cells. By month 60, the median (min, max) VCN was 0.64 (0.14, 1.51) for PBLs and 0.66 (0.14, 1.57) for CD14+ cells.

In study ALD-104, by month 1 median (min, max) VCN was 1.25 (0.69, 1.88) c/dg for PBLs and 1.59 (0.82, 2.81) c/dg for CD14+ cells. By month 6, which was the latest data cut-off point for the MA application, the median (min, max) VCN was 1.57 (1.00, 3.13) for PBLs and 1.59 (1.26, 3.82) for CD14+ cells. Thus, it appears that peripheral blood and CD14+ cells vector copy numbers were somewhat higher in study ALD-104. In both studies, high variability in PB VCN and CD14+ cells were attributable to a few subjects.

In study ALD-102 and LTF-304, the median %ALDP+ cells in peripheral blood increased from 0.87% at baseline to 18.75% at month 1 and then gradually decreased down to 4.25% at month 60, albeit with high intersubject variability. For the CD14+ cells, the median %ALDP+ cells appeared somewhat more stable, increasing from 1% at baseline to 29.5% at month 1 and still at 21.25% at month 60 (also with high inter-subject variability). Similar results were seen for the initial 6 months of study ALD-104. Since ALDP is not a secreted protein, normal values in different cell types in healthy individuals are not known. The assay for cells expressing transgenic ALDP was designed to yield cells producing normal levels of ALDP from endogenous *ABCD1* genes ALDP negative. In contrast, virtually all cells producing ALDP from the transgenic *ABCD1* cDNA will be ALDP+ because the transgenic cells over-express the transgenic ALDP. It is further not known what percentage of ALDP+ cells are needed for a beneficial effect in CALD patients. The only conclusions that may be drawn is that the majority of eli-cel treated subjects have ALDP+ in CD14+ cells in peripheral blood.

Thus, no intracellular normal values of ALDP are known and the percentage of ALDP-positive CD14+ cells of a mean 18-28% over month 1-60 after eli-cel treatment may only indicate a higher than normal presence of ALDP in the positive cells.

The applicant states that the presence of the transgene is expected to be lifelong after Eli-infusion. Detectable PB VCN up to 60 months after Eli-cel infusion, has been demonstrated, but the IQR and mean %ALDP+ cells in periphery blood seem to decline over time. Thirteen patients have been observed > 60 months. The applicant has presented updated results showing persistence of transduced HSCs over time. VCN levels do not appear to decrease extensively over time, neither in PBLs nor CD14+ cells. Results for %ALDP+ Cells are less clear and indicate a decrease at month 60 for %ALDP+ cells in PBL. Monitoring of the maintenance of efficacy is an important measure post-approval.

With regards to the effects on lowering of serum VLCFAs, it is noticed that the percent change from baseline at month 6, 12 and 24 for C26:0 LysoPC (+5%, -6%, -20%) and C26:0/C22:0 ratio (-11%, -14%, -15%) was considerably lower in study ALD-102 compared to the corresponding values from study ALD-103 TPES population (C26:0 LysoPC: -46%, -63%, -61%, C26:0/C22:0 ratio: -40%, -38%, -29%).When comparing the VLCFA results from ALD-103 it may be concluded that allo-HSCT shifted these values towards the range of female ALD carriers (C26:0/C22:0 ratio lowest value at month 12: 169 (min-max 125-342), C26:0 LysoPC lowest value at month 12: 0.05 (min-max 0.03-0.06)) and eli-cel treatment in study ALD-102 did the same, but to a lesser degree (C26:0/C22:0 ratio lowest value at month 24: 355 (min-max 161-603), C26:0 LysoPC lowest value at month 12 and 24: 0.06 (min-max 0.04-0.09)). However, neither treatment was even close to normalising the VLCFA values.

The applicant states that it is not known if there is a threshold for VLCFA toxicity and that serum levels of VLCFAs do not correlate with the clinical severity or phenotype of ALD in males. Even though 85% of heterozygote ALD women have elevated VLCFAs, still very few women develop CALD. In conclusion, the level of reduction of serum VLCFA levels do not enable a prediction of the likelihood of clinical benefit for the treated CALD subjects.

The PD markers generally displayed maintained levels over long-term follow up to month 60 in study LTF-304.

The pharmacodynamic endpoints PB VCN and %ALDP+ cells are investigated in the studies ALD-102, ALD-104 and LTF-304. However, fewer exploratory PD endpoints were investigated in ALD-104 and LTF-304. All PK/PD correlations were presented initially only for study ALD-102. The applicant provided an updated analysis with data from study ALD-104, which supported prior conclusions from study ALD-102.

In both ALD-102 and ALD-104 (although without statistical significance due to the low number of subjects for study ALD-104), DP VCN correlated both with PB VCN and %ALDP+, which is indicative of engraftment of the transduced cells. Consequently, PB VCN also correlates with %ALDP+. There was however no clear correlation of PB VCN with VLCFA levels.

No correlation was observed between cell dose and day of either neutrophil or platelet engraftment.

The applicant states that the lack of correlation of cell dose with the Day of neutrophil or platelet engraftment in Study ALD-102 suggests that even the lowest dose received (5.0×10^6 CD34+ cells/kg) is adequate for eli-cel to effectively reconstitute the haematopoietic system of subjects.

There was also no clear correlation between PD measures such as DP VCN and clinical outcome. However, it is noted that the DP VCNs were among the lowest (0.5 c/dg) for one subject in study ALD-102 who developed MFD and also for one other subject who received allo-HSCT after being discontinued form study ALD-102. For the other subject who was discontinued to receive allo-HSCT, the DP VCN was 1.2 c/dg, which corresponds to the median DP VCN for study ALD-102.

Two of the three subjects in study ALD-102 who didn't achieve MFD-free survival at month 24 had DP VCN among the lowest (0.5 c/dg) in the study. The applicant states that from the Oct 2020 data cut-off, study ALD-102 results show an 'optimal outcome' with DP VCN ≥0.7 c/dg and subjects with 'suboptimal outcome' (here defined as MFD, or study withdrawal, or NFS change ≥ 2 at any time) tended to cluster amongst subjects with the lowest PD parameters. It is noted that among the N=6 subjects with DP VCN below 0.7 c/dg in study ALD-102, the mean Loes score change from baseline at latest assessment was 8.1 (min/max: 0-18.5, median 6.75) vs a mean 4.0 change from baseline for the whole study population (N=32) in ALD-102. This is supportive of the decision in 2017 to change the DP VCN specification lower limit from 0.5 to 0.7 c/dg. This change has led to higher DP and PD data for ALD-104 than ALD-102, with the median DP VCN being 1.60 vs 1.20 and substantially higher median VCN and %ALDP+ in CD14+ cells at month 12 (1.64 vs 0.50 c/dg, 38.6% vs 17.2%). Looking at the preliminary clinical results, the superior PD data in study ALD-104 have not translated into an apparent better clinical outcome so far, as measured in median Loes score at month 12 (6.5 (N=8) vs 3.0 (N=32)). The applicant has clarified that clinical decisions with regards to follow-up or patient care / additional treatment may not be based on PD marker data, since no clear correlation between PD markers and clinical outcome has been established.

2.4.5. Conclusions on clinical pharmacology

Because of the nature of Skysona, conventional PK data based on absorption, distribution, metabolism, and excretion is not expected. Acceptable methods were developed to investigate the pharmacodynamics in terms of vector copy number, ALDP protein expression and for safety, the detection of replication competent lentivirus. The conditioning regimens were also found acceptable.

The applicant has shown that about 50% of CD34+ cells in the drug product are transduced with the transgene in both clinical studies on eli-cel (ALD-102 and ALD-104). Furthermore, the transgene is localised in peripheral blood leukocytes and CD14+ cells, the latter corresponding to the CD14+ cells migrating to the CNS and contributing to the therapeutic effects of eli-cel. However, it has not been convincingly demonstrated that this leads to a clinically relevant increase in % of ALDP+ cells or lowering of VLCFAs in plasma, nor is it known to which extent plasma levels correspond to levels in the CNS. Thus, the PD results confirm that the drug product contains the transgene, but no relation to clinical effects have been demonstrated.

2.5. Clinical efficacy

The main evidence of efficacy comes from study ADL-102. Data from the ongoing study ALD-104 and long term follow up ALD-304 support the safety and efficacy of the product. Studies ALD-101 and ALD-103 are historical control studies describing disease progression and neurological decline in untreated CALD patients or allo-HSCT treated patients.

Table 4 Overview of Clinical Studies Evaluating Eli-cel and Allo-HSCT in Subjects With CALD

Study Identifier (Status); Location of CSR or Protocol (as applicable)	Study Title	Age, Number of Subjects and Treatment Performed	Conditioning Regimen	Primary Efficacy Endpoint	Data Cut
Eli-cel Treatment and Long-term Follow-up					
ALD-102 (ongoing) 5.3.5.2 Interim CSR ALD-102	A Phase 2/3 Study of the Efficacy and Safety of Hematopoietic Stem Cells Transduced With Lenti-D Lentiviral Vector for the Treatment of Cerebral Adrenoleukodystrophy (CALD)	Males <18 y.o. 30 planned 32 treated with eli-cel	busulfan (IV) and cyclophosphamide (IV)	Proportion of subjects who are alive, and have none of the 6 MFDs at Month 24 Visit (i.e. Month 24 MFD-free survival)[a]	Interim Data Cut: 17 January 2020
ALD-104 (ongoing) 5.3.5.2 Interim CSR ALD-104	A Phase 3 Study of Lenti-D Drug Product After Myeloablative Conditioning Using Busulfan and Fludarabine in Subjects ≤17 Years of Age With Cerebral Adrenoleukodystrophy (CALD)	Males <18 y.o. 35 planned 13 treated with eli-cel	busulfan (IV) and fludarabine (IV)	Proportion of subjects who are alive, and have none of the 6 MFDs at Month 24[a]	Interim Data Cut: 21 February 2020
LTF-304 (ongoing) 5.3.5.2 Interim CSR LTF-304	Long-term Follow-up of Subjects With Cerebral Adrenoleukodystrophy Who Were Treated With Lenti-D Drug Product	Long-term follow up for all subjects with CALD who received eli-cel in parent studies 21 enrolled (21 from Study ALD-102, 0 from study ALD-104)	Not applicable (Subjects are not treated with eli-cel in this long-term follow-up study)	MFD-free survival	Interim Data Cut: 31 January 2020
Untreated or Allo-HSCT					
ALD-103 (completed) (Sponsor terminated study after 59 subjects were enrolled and analyzed) 5.3.5.4 Final CSR ALD-103	A Prospective and Retrospective Data Collection Study to Evaluate Outcomes in Males ≤17 Years of Age Undergoing Allogeneic Hematopoietic Stem Cell Transplantation for the Treatment of Cerebral Adrenoleukodystrophy	Males <18 y.o. 60 planned 59 treated with allo-HSCT	Investigator determined as per institutional guidelines	Evaluate the efficacy of allo-HSCT in subjects with CALD	Database Lock 31 March 2020
ALD-101 (completed) 5.3.5.4 Final CSR ALD-101	A Retrospective Study to Characterize the Natural History of Childhood Cerebral X-linked Adrenoleukodystrophy and to Investigate the Influence of Allogeneic Transplantation on Affected Subjects	Inclusion criterion: Males >3 and <15 y.o Enrolled: Males >1 and <15 y.o. 137 subjects: 72 untreated 65 treated with allo-HSCT	Not applicable (untreated) or Investigator's discretion (allo-HSCT treated)	Characterize the natural history of disease in untreated subjects with CALD, and to characterize the efficacy and safety outcomes of subjects with CALD who are treated with allo-HSCT, for the purpose of defining efficacy and safety endpoints useful for the design of clinical studies.	Database Lock: 27 March 2012

2.5.1. Dose-response studies

No dose-response study was performed.

Dosing rationale:

The dose of eli-cel drug product is a single intravenous dose of ≥ 5.0 x 10^6 CD34+ cells/kg patient weight.

The applicant states that the minimum CD34+ dose accepted as safe practice and associated with favourable engraftment kinetics in allo-HSCT is approximately 1.5 × 10^6 to 3.0 × 10^6 CD34+ cells/kg.

However, optimal neutrophil and platelet engraftment has been reported to occur at doses around 5.0 × 10^6 CD34+ cells/kg.

To date, a total of 45 subjects (32 subjects in Study ALD-102 and 13 subjects in Study ALD-104) have received doses of 5.0 to 38.2 × 10^6 CD34+ cells/kg, and the lack of correlation of cell dose with the time to neutrophil or platelet engraftment suggests that even the lowest dose received (5.0 x 10^6 CD34+ cells/kg) is adequate for eli-cel to effectively reconstitute the haematopoietic system of subjects. Thus ≥ 5.0 x 10^6 CD34+ cells/kg is the recommended dose for eli-cel.

No upper dose limit is specified. For subject safety, only the minimum number of mobilisation/apheresis cycles are performed to collect sufficient cells required for drug product manufacturing to meet the minimum dose. Therefore, it is not practicable to determine a maximal tolerable cell dose. To the applicant's knowledge, there is no maximum recommended dose of CD34+ cells for infusion of autologous cell products in the literature. Adverse events due to high CD34+ cell dose have not been reported in studies with allo-HSCT or in eli-cel clinical studies.

2.5.2. Main study

Pivotal study ALD-102

Study ALD-102 is an international multi-site, non-randomised, open label, single-dose, single-arm, prospective phase 2/3 study in male subjects with CALD treated with eli-cel.

The study is ongoing; it started in 2013 and the Interim study report with the Data cut-off: 17 January 2020 was produced for the Marketing authorisation application.

Methods

Study participants

Male subjects aged 17 years and younger with active cerebral ALD as defined by elevated VLCFA values and active central nervous system (CNS) disease established by central radiographic review of brain MRI demonstrating Loes score between 0.5 and 9 (inclusive) on the 34-point scale, and Gadolinium enhancement (GdE+) on MRI of demyelinating lesions. The neurologic function scale (NFS) of ≤1. The main exclusion criterion was availability of a willing 10/10 HLA-matched sibling donor (excluding female heterozygotes).

Treatments

Mobilisation and Apheresis

Subjects were mobilised with G-CSF for 4 to 6 days.

Apheresis was performed per standard clinical site practice. Up to 3 total collections could be performed as part of any one mobilisation cycle until the target of 12 × 10^6 CD34+ cells/kg was obtained for transduction.

Conditioning

Pre-conditioning assessments were performed 11 days prior to eli-cel infusion (defined as Rel Day -11; with a window of -3 days, i.e. Rel Day -14 through Rel Day -11) prior to myeloablative conditioning. If neurological decline was observed, as evidenced by a NFS > 1 or a Loes Score > 9, the subject was discontinued from the study.

Conditioning only began once eli-cel was dispositioned for clinical use and the drug product was at the clinical site. Myeloablative and lymphodepleting conditioning was performed on an in-patient basis using first busulfan IV followed by cyclophosphamide IV.

Weight-based dosing of busulfan IV was administered on Rel Days -10, -9, -8, and -7 and weight-based dosing of cyclophosphamide IV was administered on Rel Days -5, -4, -3, and -2.

Eli-cell treatment

Infusion of eli-cel was given approximately 48 hours after the last dose of cyclophosphamide. The dose administered was ≥ 5.0×10^6 CD34+ cells/kg.

Prohibited concomitant therapies

Medications used to lower VLCFA levels (e.g., Lorenzo's oil, statins) and other investigational therapies were excluded during study participation. In addition, subjects were not to start a new low-fat diet during study participation. There were no other excluded concomitant medications or vaccines.

Objectives

The objective of the current study was to demonstrate that treatment with eli-cel is able to stabilize the disease at a level of neurological function that preserves the capacity for independent living for subjects with CALD, i.e. that the patients didn't develop any major functional disabilities (MFDs).

The second objective of this study was to evaluate the safety of eli-cel in subjects with CALD.

Success criterion

In accordance with prior Regulatory Agency advice, the success criterion for this study was based on a comparison of the ALD-102 primary efficacy endpoint for the Initial Cohort to a clinically meaningful benchmark, such that the lower bound of the 2-sided 95% exact CI of Month 24 MFD-free survival must be > 50% in order for the primary endpoint to be met.

This clinically meaningful benchmark of 50% is supported by the Study ALD-101 untreated study population, who were GdE+ at any time, as well as data from the ALD-101 allo-HSCT treated population, now published in the literature (Raymond et al., 2018). There is additional supportive context from disease-specific literature that reports on overall survival rather than MFD-free survival (Baumann et al., 2003; Beam et al., 2007; Miller et al., 2011; Peters et al., 2004). As patients can and do progress through MFDs to death, the results of these studies can be further informative to this benchmark. In Study ALD-101, the Month 24 MFD-free survival rate in untreated GdE+ subjects within 2 years of their first GdE+ MRI was 21% (exact 95% CIs of 6.1% to 45.6%). Thus, this benchmark value is above the upper bound of the 95% CI of the Month 24 MFD-free survival in untreated subjects in Study ALD-101.

In Study ALD-101, the lower bound of the 2-sided 95% exact CI of the Month 24 MFD-free survival rate in GdE+, early disease (NFS≤1, Loes ≥0.5 and ≤9) subjects without a matched sibling donor and treated with allo-HSCT was 50.1% (mean 76% with exact 95% CI of 50.1% to 93.2%).

An MFD-free survival rate at various timepoints ranging between approximately 50% to 90% is reported in existing literature for patients with CALD treated with allo-HSCT. Peters et al. reported that 53.4% of subjects treated with early clinical disease had no neurological progression and remained functional; the 5-year survival for subjects with 0 to 1 clinical symptoms at baseline was 67% to 70% (Peters et al., 2004). Miller et al. reported a 5-year survival rate of 91% (95% CI 69% to 98%) for patients with a baseline NFS of 0, and 89% (95% CI 70% to 96%) for patients with a baseline Loes score <10. The follow-up median NFS for both cohorts was 0, with an interquartile range of 0 to 0 (NFS of 0) and 0 to 1 (Loes score <10) (Miller et al., 2011). Both of these studies included subjects

with matched sibling donors; however, subjects with a willing 10/10 HLA-matched sibling donor are excluded from the ALD-102 study.

Similar rates are reported in two smaller studies; Beam et al and Bauman et al. Beam et al. reported an overall survival of 73% after approximately 4 years of follow-up post-allo-HSCT. Of the 6 patients who had baseline Loes scores ≤9, 5 (83%) patients were alive and functional (Beam et al., 2007). Baumann et al. reported 42% functional survival after approximately 3.7 years of follow-up. Of the 7 patients who had baseline Loes scores of ≤9 and GdE+, 6 (85.7%) patients were alive and functional (Baumann et al., 2003). The subjects in these studies had similarities to the ALD-102 study population: both Beam et al. and Baumann et al. reported on subjects with baseline Loes scores ≤9 and the subjects in the Baumann comparison were gadolinium positive at baseline. Thus, a mean MFD-free survival rate greater than 70% would be consistent with the statistics reported in the literature.

Outcomes/endpoints

Primary efficacy endpoint:

Proportion of subjects who were alive and have none of the 6 major functional disabilities (MFDs) at Month 24 Visit (i.e. Month 24 MFD-free survival). MFDs were defined as:

- o loss of communication
- o cortical blindness
- o tube feeding
- o total incontinence
- o wheelchair dependence
- o complete loss of voluntary movement

In addition to experiencing any MFDs or death, the following events were also considered as a failure to meet the primary efficacy endpoint: requirement for rescue cell administration or an allo-HSCT, withdrawal from study, or lost to follow-up by Month 24.

Secondary efficacy endpoints included MFD-free survival over time**,** Overall survival, Proportion of subjects who demonstrated resolution of gadolinium positivity on magnetic resonance imaging (MRI; i.e., GdE-) at Month 24**,** Time to sustained resolution of gadolinium positivity on MRI (i.e., GdE-). Sustained is defined as gadolinium resolution without a subsequent evaluation indicating gadolinium positivity**,** Change in total Neurologic Function Score (NFS) from Baseline to Month 24

Exploratory efficacy endpoints at Month 24 included Proportion of subjects who maintained an NFS ≤ 4 without an increase of > 3 points from Baseline**,** Change in Loes score from Baseline**,** Proportion of subjects who maintained a Loes score ≤ 9 or did not increase their Loes score by≥ 6 points from Baseline**,** Change from Baseline to Month 24 in neuropsychological tests (data not presented)**,** Pediatric Quality of Life Inventory (PedsQL) score, Global Assessment Scores

Neurologic Function Score (NFS):

Hearing/auditory processing problems	**1**
Aphasia/apraxia	**1**
Loss of communication	**3**
Vision impairment/fields cut	1
Cortical blindness	2
Swallowing difficulty or other central nervous system dysfunction	**2**
Tube feeding	**2**
Running difficulties/hyperreflexia	1
Walking difficulties/spasticity/spastic gait (no assistance)	1
Spastic gait (needs assistance)	2
Wheelchair required	2
No voluntary movement	3
Episodes of urinary or fecal incontinency	**1**
Total urinary or fecal incontinency	**2**
Nonfebrile seizures	1
Possible Total	**25**

Loes score:

The Loes score is a 34-point scale used to evaluate and describe disease burden throughout the brain in patients with CALD by scoring MRI findings (Loes et al. 1994).

- **Sample size**

The number of subjects planned to be infused with Lenti-D Drug Product is approximately 30. The sample size was not determined by formal statistical methods.

The study was initially designed to enrol up to 15 subjects in order to obtain at least 12 evaluable subjects. As of October 2015, under protocol versions 2.0-6.2, there were 17 subjects enrolled and treated with Lenti-D Drug Product. Analysis of these 17 subjects will be the basis for determining the success or failure of the study.

- **Randomisation, Blinding (masking)**

N/A, this was a single-armed open-label trial.

- **Statistical methods**

Statistical analyses follow the statistical analysis plan (SAP) Version 2.0, dated 31 October 2019. Data cut for the interim report is 17 January 2020.

Statistical methods were primarily descriptive and included point estimates and 2-sided 95% confidence intervals (CI) as appropriate. Tabulations and figures were produced for one or both of the cohorts below:

- Initial Study Cohort: includes the first 18 subjects screened in ALD-102 (under protocol versions 2.0-6.2). Within this cohort, 1 subject failed screening and did not receive any study treatment, while 17 subjects enrolled in the study and were treated with Lenti-D Drug Product.
- Month 24 Evaluable Subjects for MFD-free Survival: defined as subjects treated with Lenti-D Drug Product and have been followed for 24 months (Rel Day of last contact (DLC) ≥730), or have completed the Month 24 visit, or had discontinued from the study but would have been followed for 24 months (Rel Day of data cut ≥730) if still in the study, at the time of the data cut for initial analysis. The 17 treated subjects in Initial Study Cohort constitute part of the Month 24 Evaluable Subjects.
- Overall Study Cohort: includes all screened subjects at the time of the data cut. This cohort includes the Initial Study Cohort as well as the additional subjects who were screened afterwards.

Planned analyses for this study include the following:

- Initial Analysis: to be performed after the Initial Study Cohort (enrolled under protocol versions 2.0-6.2) complete the study or when the sponsor deems appropriate. This analysis includes all data from Initial Study Cohort, Month 24 Evaluable Subjects for Month 24 MFD-free Survival, and the Overall Study Cohort, at the time of the data cut.
- Final Analysis: to be performed when all subjects treated with Lenti-D Drug Product complete the study.

Analysis populations were following:

- The Intent-to-treat population (ITT) consisted of subjects who initiate any study procedures, beginning with stimulation by G-CSF.
- The Transplant population (TP) consisted of subjects who received Lenti-D Drug Product.
- The Successful Neutrophil Engraftment Population (NEP) consisted of subjects who achieved neutrophil engraftment defined as having 3 consecutive ANC laboratory values of $\geq 0.5 \times 10^9$ cells/L (after initial post-infusion nadir) obtained on different days by 42 days post-infusion of Lenti-D Drug Product.

Because the ITT, TP, and NEP populations were identical in this interim analysis, all results are reported for the TP, also referred to as the "Overall Cohort TP". Some results are also presented for the "Initial Cohort TP", defined as the first 17 subjects treated with drug product.

The primary efficacy endpoint is the proportion of subjects who have success in Month-24 MFD-free survival, which is a binary endpoint. To be considered a success for the primary endpoint, a subject must meet all following criteria:

1. Be alive at the Month 24 Visit.
2. Have not developed any of the MFDs by the Month 24 Visit.
3. Have not received rescue cell administration or allo-HSCT by the Month 24 Visit.
4. Have not withdrawn from the study or been lost to follow-up by the Month 24 Visit.

For the primary analysis, the number and percent of subjects who achieve Month 24 MFD-free survival was presented with the exact 95% CI (obtained using the Clopper-Pearson method) for the TP in the Initial Study Cohort and in the cohort of the Month-24 Evaluable Subjects for MFD-free Survival (the data cut for the Initial Analysis). The lower bound of the 2-sided 95% exact confidence interval of the Month 24 MFD-free survival rate must be >50% in order for the primary endpoint to be met.

For a sensitivity analysis, failures for Month-24 MFD-free survival only included failures that occur on or before Rel Day 730. This sensitivity analysis was only to be conducted when it is different from the primary analysis. Number and percent of subjects with failure was reported with 2-sided 95% CI.

The analyses of the secondary and exploratory efficacy endpoints were performed on the TP. The time-to-event analysis of MFD-free survival and overall survival was based on the Kaplan-Meier methodology. Event rates and 95% CIs at 12 and 24 months, respectively, post Lenti-D Drug Product infusion (DPI), and the restricted mean survival time (RMST) at 24 months post DPI were also presented. For the endpoints stable NFS at Month 24 and stable Loes score at Month 24, evaluable subjects are defined as subjects who have non-missing Baseline values and have non-missing Month 24 assessment results for the corresponding parameter.

The primary safety endpoint was the proportion of subjects who have experienced either acute (≥Grade II) or chronic GVHD at Month 24 in the TP population. It was designated the primary safety endpoint mainly for comparisons with CALD subjects who received allo-HSCT, particularly in studies ALD-101 and ALD-103, which was detailed in the inter-study SAP. The general safety profile of treatment with Lenti-D Drug Product was summarised through the longitudinal evaluation of AEs, laboratory assessments, vital signs, ECG and physical examination findings. Safety parameters were summarised across each time point.

In general, there were no substitutions made to accommodate missing data points. Subjects who discontinued prior to the Month 24 visit were considered treatment failures in the primary efficacy analysis. No multiplicity adjustment was made in the primary analysis as it was performed on the initial cohort as prespecified.

A separate inter-study SAP describes comparisons of data from this study and its long-term extension Study LTF-304 with data from a retrospective study of CALD (Study ALD-101) and an observational study of allo-HSCT-treated patients with CALD (Study ALD-103).

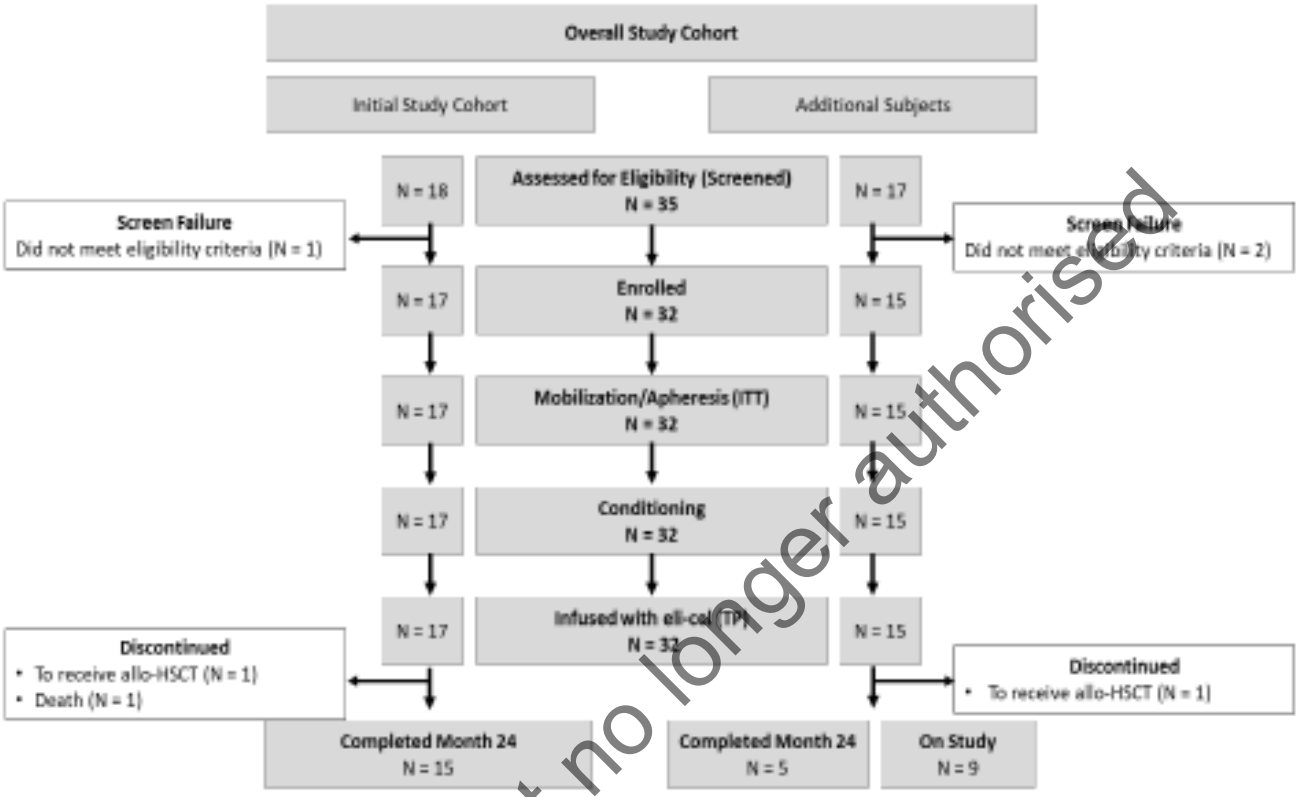
Changes of the analyses

The study was initially designed to enrol up to 15 subjects in order to obtain at least 12 evaluable subjects. As of October 2015, under protocol versions 2.0-6.2, there were 17 subjects enrolled and treated with Lenti-D Drug Product as well as 1 screen failure. Analysis of these 17 subjects will be the basis for determining the success or failure of the study. In order to obtain experience with the European contract manufacturing organisation and to continue to provide a treatment option for patients who could benefit from Lenti-D Drug Product in a controlled setting, the Study protocol was later amended to treat approximately 30 subjects. Despite the addition of this second cohort of subjects, the nature of the primary analysis (based on the cohort of the first 17 treated subjects) has not changed. A final analysis will be performed as supportive when all subjects complete the study.

Results

- **Participant flow**

Figure 2 Study Participant flow



Subjects visited the same site (the primary site) for Screening through treatment, Month 12, and Month 24 assessments, but subjects were permitted to visit sites (secondary sites) that were closer to home for other study visits. For the Initial Cohort, all primary sites were in the US and sites in Australia, Argentina, France, and UK were open only as secondary sites. After the Initial Cohort, sites in France and the UK were open as primary sites and Germany was added as a primary site.

Baseline data

Table 5 Subject Demographics (TP)

	Initial Cohort (N = 17)	Overall Cohort (N = 32)
Age at ICF (years)		
Median	6.0 (5.0, 7.0)	6.0 (4.0, 7.0)
Minimum, Maximum	4, 13	3, 13
Age at ICF category, (years) n (%)		
≥ 2 to < 6		
≥ 6 to < 12		
≥ 12 to < 18		
Age at diagnosis of CALD (years)		
Median	6.0 (4.0, 6.0)	6.0 (4.0, 7.0)
Minimum, Maximum	3, 13	1, 13
Age at eli-cel infusion (years)		
Median	6.0	6.0
Minimum, Maximum	4, 14	4, 14
Gender		
Male	17 (100.0)	32 (100.0)
Race, n (%)		
White	9 (52.9)	15 (46.9)
Black or African American	0	1 (3.1)
Asian	0	1 (3.1)
Other	3 (17.6)	5 (15.6)
Not Reported	5 (29.4)	10 (31.3)
Ethnicity, n (%)		
Hispanic	7 (41.2)	12 (37.5)
Non-Hispanic	8 (47.1)	17 (53.1)
Not Reported	2 (11.8)	3 (9.4)

Source: Table 14.1.3.1

Abbrev.: ICF, time of signing informed consent form; CALD, cerebral adrenoleukodystrophy; TP, transplant population

	Initial Cohort (N = 17)	Overall Cohort (N = 32)
Age at ICF (years)		
Median	6.0 (5.0, 7.0)	6.0 (4.0, 7.0)
Minimum, Maximum	4, 13	3, 13
Age at diagnosis of CALD (years)		
Median	6.0 (4.0, 6.0)	6.0 (4.0, 7.0)
Minimum, Maximum	3, 13	1, 13
Age at eli-cel infusion (years)		
Median	6.0	6.0
Minimum, Maximum	4, 14	4, 14
Gender		
Male	17 (100.0)	32 (100.0)
Race, n (%)		
White	9 (52.9)	15 (46.9)
Black or African American	0	1 (3.1)
Asian	0	1 (3.1)
Other	3 (17.6)	5 (15.6)
Not Reported	5 (29.4)	10 (31.3)
Ethnicity, n (%)		
Hispanic	7 (41.2)	12 (37.5)
Non-Hispanic	8 (47.1)	17 (53.1)
Not Reported	2 (11.8)	3 (9.4)

Source: Table 14.1.3.1
Abbrev.: ICF, time of signing informed consent form; CALD, cerebral adrenoleukodystrophy; TP, transplant population

Table 6 Baseline Disease Characteristics (TP)

	Initial Cohort (N = 17)	Overall Cohort (N = 32)
Family history, n (%)	11 (64.7)	19 (59.4)
Method of diagnosis[a], n (%)		
VLCFA Testing	16 (94.1)	29 (90.6)
ABCD1 Genotyping	10 (58.8)	21 (65.6)
MRI with Gadolinium Contrast	13 (76.5)	23 (71.9)
Signs and symptoms, n (%)	17 (100.0)	31 (96.9)
Adrenal insufficiency	14 (82.4)	27 (84.4)
Seizures	0	1 (3.1)
Gait disturbance	1 (5.9)	1 (3.1)
Other	2 (11.8)	3 (9.4)
Baseline Neurologic Function Score		
0	17 (100.0)	31 (96.9)
1	0	1 (3.1)
Baseline Loes score		
Median	2.00	2.00
Minimum, Maximum	1.0, 7.5	1.0, 9.0
Baseline Loes pattern, n (%)[b]		
Pattern 3 and/or 4 only	2 (11.8)	3 (9.4)
Patterns include 1, 2, 5[c]	15 (88.2)	29 (90.6)
Time from informed consent to eli-cel infusion (days)		
Median	67.0	67.0
Minimum, Maximum	58, 89	58, 89
Time from diagnosis of CALD to eli-cel infusion (months)		
Median	5.82	5.83
Minimum, Maximum	2.5, 17.2	2.5, 26.8

Source: Table 14.1.3.2

Abbrev.: CALD, cerebral adrenoleukodystrophy; MRI, magnetic resonance imaging; TP, transplant population; VLCFA, very long-chain fatty acids

[a] More than one method of diagnosis may have been used per subject.

[b] Loes Patterns: 1 = Parietal-occipital; 2 = Frontal; 3 = Pyramidal tracts involvement; 4 = Cerebellar white matter involvement; 5 = Combined parieto-occipital and frontal white matter involvement.

[c] Patterns include 1,2,5. Subjects with this pattern may also have 3, 4 or other.

- **Numbers analysed**

Table 7 Disposition (ITT)

	Statistic	Initial Cohort (N = 17)	Overall Cohort (N = 32)
Initiated mobilization (ITT)	n (%)	17 (100.0)	32 (100.0)
Initiated conditioning	n (%)	17 (100.0)	32 (100.0)
Infused with eli-cel (TP)	n (%)	17 (100.0)	32 (100.0)
Successful neutrophil engraftment (NEP)[a]	n (%)	17 (100.0)	32 (100.0)
Completed Study		15 (88.2)	20 (62.5)
Discontinued Study	n (%)	2 (11.8)	3 (9.4)
Reasons for study discontinuation			
Death	n (%)	1 (5.9)	1 (3.1)
Subject to receive allo-HSCT	n (%)	1 (5.9)	2 (6.3)
Enrolled in Study LTF-304[b]	n (%)	15 (88.2)	20 (62.5)
In study at data cut	n (%)	0	9 (28.1)
Duration of follow-up (months)	Median	23.85	23.62
	Min., Max.	13.4, 25.3	9.1, 25.3
Subject-years of follow-up (years)[c]		33.0	58.7
Latest Visit Completed			
Month 9	n (%)	0	1 (3.1)
Month 12	n (%)	1 (5.9)	1 (3.1)
Month 15	n (%)	0	1 (3.1)
Month 18	n (%)	0	2 (6.3)
Month 21	n (%)	1 (5.9)	7 (21.9)
Month 24	n (%)	15 (88.2)	20 (62.5)

Source: Table 14.1.1.2, Table 14.1.2, Table 14.1.1.4

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; ITT, intent-to-treat population; NEP, neutrophil engraftment population; TP, transplant population

[a] The Successful Neutrophil Engraftment Population (NEP) consists of subjects who achieved neutrophil engraftment defined as having 3 consecutive absolute neutrophil count (ANC) laboratory values of $\geq 0.5 \times 10^9$ cells/L (after initial post-infusion nadir) obtained on different days by 42 days post-infusion of eli-cel (Relative Day 43).

[b] LTF-304 is the long-term follow-up study to support parent eli-cel studies.

[c] Subject-years were calculated by summing the total of the number of years each subject has been followed after drug product infusion.

The ITT, TP, and NEP populations were identical. Therefore, all analyses were performed on the TP (also referred to as the "Overall Cohort TP"). Some analyses were also performed on the first 17 eligible subjects who enrolled (also referred to as the "Initial Cohort TP"), as per planned analyses.

- **Outcomes and estimation**

Pharmacodynamic outcomes

Presented in the PD section above.

Efficacy

The study success criterion for efficacy: Month 24 MFD-free survival

Success criterion for efficacy was based upon having a lower 95% CI of > 50.0% for the endpoint of Month 24 MFD-free survival for the Initial Cohort. This was based on comparison of the primary efficacy endpoint results for the Initial Cohort TP (N = 17) to a clinically meaningful benchmark.

Table 8 MFD-Free Survival at Month 24 (TP)

	Initial Cohort (N = 17)	Overall Cohort (N = 32)
Month 24 evaluable subjects[a]	17	23
Month 24 MFD-free survival[b]		
n (%)	15 (88.2)	20 (87.0)
Exact 95% CI[c]	(63.6, 98.5)	(66.4, 97.2)
Month 24 MFD-free survival, sensitivity analysis[d]		
n (%)	16 (94.1)	22 (95.7)
Exact 95% CI[c]	(71.3, 99.9)	(78.1, 99.9)
Initial failure of MFD-free survival by Month 24, n (%)		
MFD	1 (5.9)	1 (4.3)
Allo-HSCT	1 (5.9)	2 (8.7)

Source: Table 14.2.1.1

Abbrev.: allo-HSCT, allogeneic stem cell transplantation; MFD, major functional disability

[a] Evaluable subjects are defined as subjects who have been followed for 24 months (i.e. Rel DLC ≥ 730) or have completed the Month 24 Visit, or discontinued from the study but would have been followed for 24 months if still on the study (i.e. Rel Day of data cut ≥ 730), at the time of the data cut.

[b] Month-24 MFD-Free survival criteria defined as: alive at 24 months post infusion; have not developed any of the MFDs by 24 months post infusion; have not received rescue cell administration or allo-HSCT by 24 months post infusion; and have not withdrawn from the study or lost to follow-up by 24 months post infusion.

[c] Exact 95% CIs is obtained using the Clopper-Pearson method.

[d] In sensitivity analysis, only death and MFDs are considered failures.

Initial Cohort

In the Initial Cohort, 15/17 or 88.2 % (95% CI 63.6, 98.5) of subjects infused with eli-cel met the primary endpoint (Month 24 MFD-free survival).

Because the lower bound (63.6%) is above the threshold of 50%, the study efficacy success criterion was met.

In the Initial Cohort, 2/17 subjects failed to achieve the primary endpoint:

One subject developed 4 MFDs (total incontinence at month 9, cortical blindness and loss of communication at month 12, and wheelchair dependence at month 21). This subject subsequently died at month 21 from disease progression.

Another subject did not develop any MFDs but was withdrawn from the study at month 13 post-treatment to undergo an allo-HSCT due to increased Loes score (+7), NFS (+1) and re-emergence of contrast enhancement on MRI (GdE+). At Baseline, this subject had a Loes score of 2.0, NFS of 0, was GdE+, and had a Loes Pattern 3 (associated with slower progression). At last evaluation in Study ALD-102 before allo-HSCT, he had a Loes Score of 9.0, NFS of 1, was GdE+, and had Loes Pattern 2 (associated with faster progression). Both subjects were treated at the same site, this subject was

withdrawn a few months after the first subject noted above had his first MFD, thus the applicant states that it is possible that the rapid decline of the first subject contributed to the decision to remove this second subject from the study.

Overall Cohort

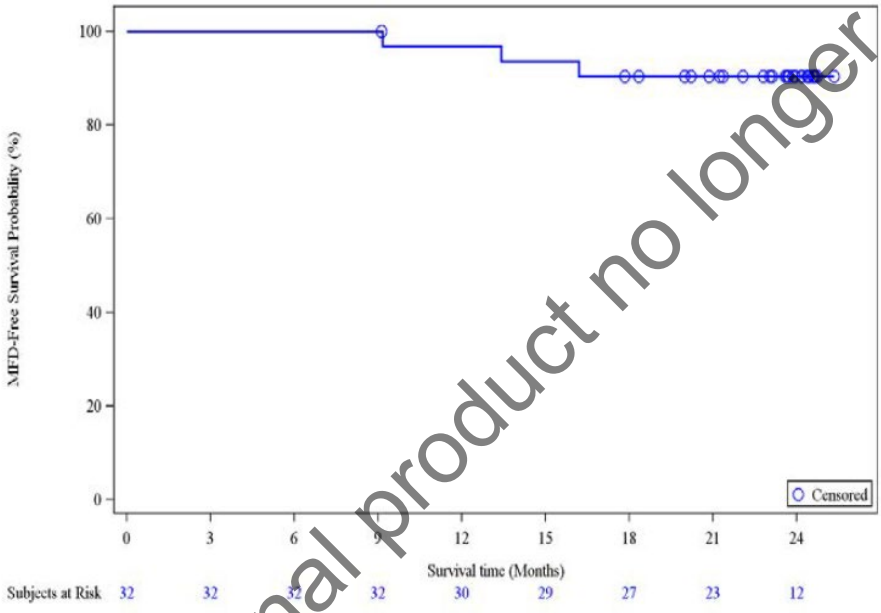
In the Overall Cohort, 20/23 or 87.0% (95% CI: 66.4, 97.2) of evaluable subjects infused with eli-cel also met the primary endpoint (Month 24 MFD-free survival), with the lower bound (66.4%) that is above the threshold of 50%. Meeting this pre-specified clinical benchmark indicates that treatment with eli-cel has a significant clinical benefit over no treatment.

In the Overall Cohort, 3/23 subjects failed to achieve the primary endpoint, including the 2 subjects in the Initial Cohort described above and an additional subject who was withdrawn by the investigator at month 16 due to increased Loes score (from 1 to 10, stable NFS at 0 and GdE-) to receive allo-HSCT.

Secondary Efficacy Endpoints:

MFD-free Survival Over Time

Figure 3 Kaplan-Meier Curve for MFD-Free Survival (TP)

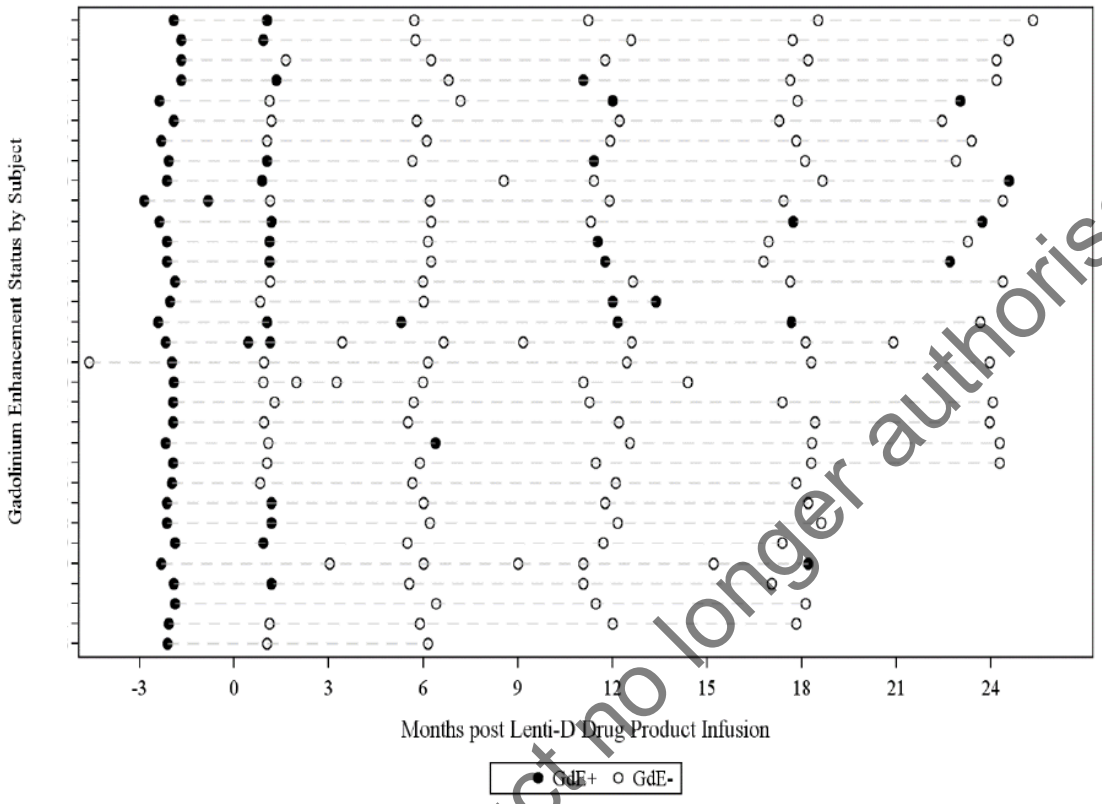


Overall survival

1 subject of 32 (described above) died 22 months after eli-cel treatment.

Contrast enhancement

Figure 4 Contrast Enhancement Status Over Time by Subject (TP)



All subjects were GdE+ on study entry (inclusion criterion #3), and GdE status was evaluated approximately every 3 months after drug product infusion.

Contrast enhancement is a subjective determination by the neuroradiologist reading the MRI. In an attempt to limit the problem of subjectivity, all MRIs were read blindly by a central reader (Dr Loes). All subjects who had completed at least their Month 6 Visit became GdE- at some time after drug product infusion. However, GdE+ signal re-emerged intermittently in several subjects treated with eli-cel, although it was frequently designated as "faint", "weak", or a "difficult call". The clinical significance of re-emergence of GdE+ in subjects treated with eli-cel is unknown. There was no apparent association between GdE status post-infusion and clinical outcome in this study.

Among the 3 treatment failures, one subject as noted above, who had rapid disease progression and died on study, was GdE- on all MRIs after his Month 1 Visit through his final MRI at Month 21 before death. Of the two subjects who withdrew to receive allo-HSCT, one was GdE+ at his last 2 MRIs before withdrawal, and the other was consistently GdE- for the last 6 MRIs before withdrawal. Seventeen out of 21 (81.0%) Month 24 evaluable subjects in the Overall Cohort were GdE- at Month 24. All 4 of the subjects who were GdE+ at Month 24 had stable NFS and also had stable Loes scores ranging from 2 to 8 at Month 24.

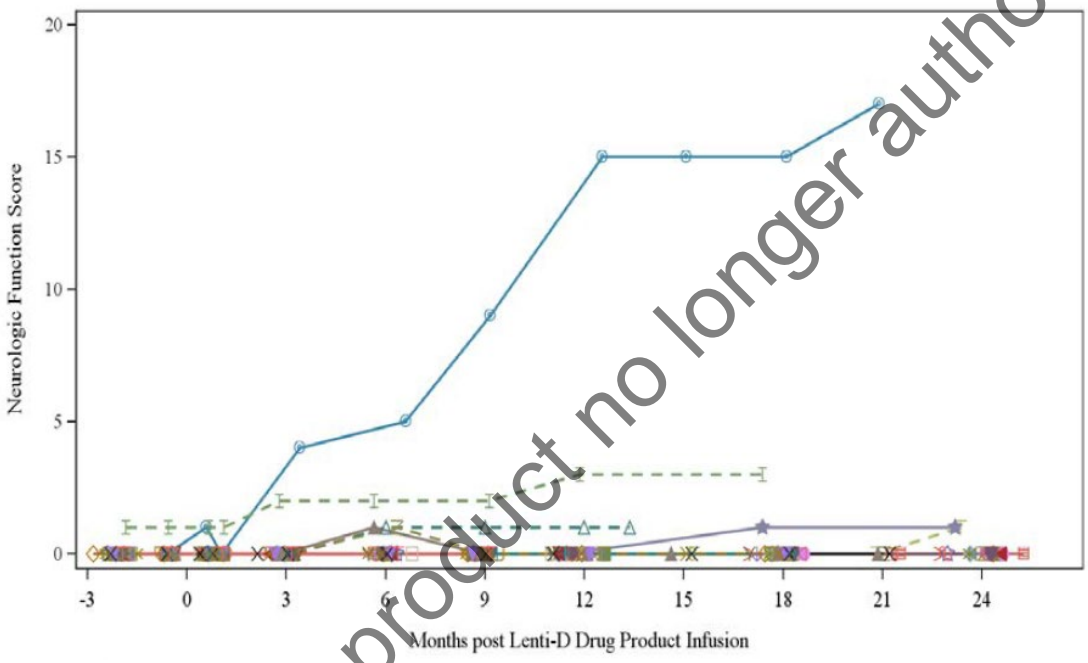
The time to sustained resolution of contrast enhancement (sustained GdE-) was analyzed for all subjects. Sustained resolution is defined as having at least two consecutive GdE- results by MRI without a subsequent evaluation indicating GdE+. Sixteen of 21 (76.2%) subjects had sustained GdE-

by Month 24. The median (min, max) Rel Day of first GdE- for those who achieved sustained GdE- was Rel Day 44.5 (29, 551).

Change in Total NFS from Baseline to Month 24

The majority of evaluable subjects (evaluable for Month 24) in the Overall Cohort (20/23, 87.0%) had an NFS of 0 at end of study Month 24 Visit. 20/23 (87.0%) evaluable subjects showed no change in NFS. 2/23 (8.7%) evaluable subjects had an increase in NFS of ≤3, resulting in an NFS of 1 at Month 24, which was due to episodes of incontinence in one subject and vision impairment in the other. 1 subject showed a rapid increase in NFS starting at Month 3. By Month 22 he had an NFS of 17 including 4 MFDs (loss of communication, cortical blindness, wheelchair dependence, and total incontinence) and he subsequently experienced cardiorespiratory arrest and died. These 3 subjects with an increase in NFS were all in the initial cohort.

Figure 5 NFS Over Time by Subject (TP)



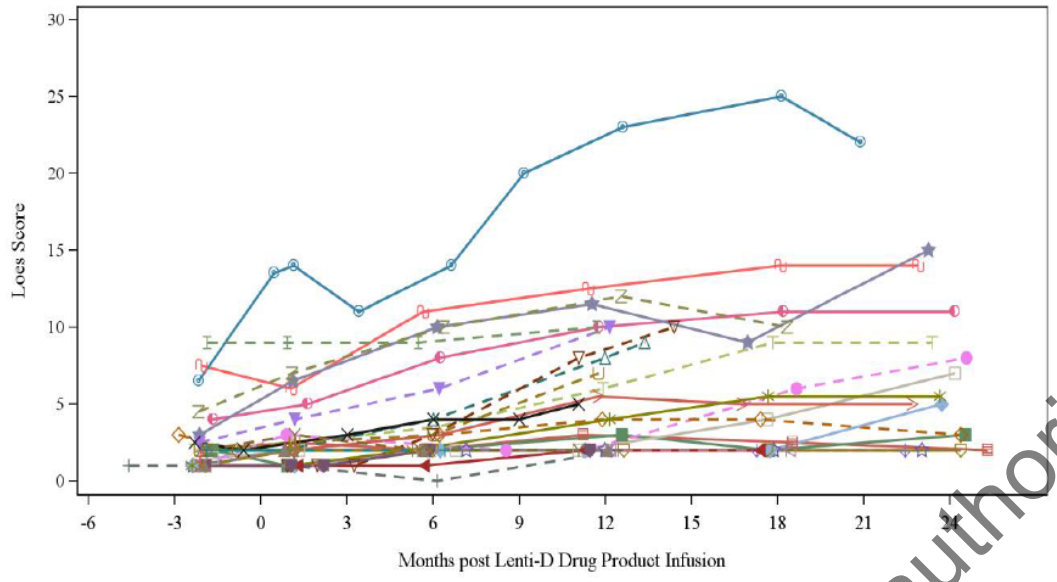
Exploratory Efficacy Endpoint:

Loes Score

21 subjects were evaluable for Month 24 Loes endpoints.

Sixteen of the 21 (76.2%) evaluable subjects had a stable Loes score at Month 24, defined as maintaining a Loes score ≤ 9 or not increasing their Loes score by ≥ 6 points since Baseline; 10 of these subjects had a change of 0 or 1.

Figure 6 Loes Score Over Time by Subject (TP)



Eight subjects had a Loes score > 9, the cut-off for inclusion in this study, at their last follow-up visit. For some of these 8 subjects, changes in Loes score were observed at the Month 1 Visit, but the majority of changes occurred from Month 6 to Month 18 with some changes presenting later at Month 24.

Table 9 Change from Baseline to Month 24 for Select Efficacy Endpoints Stratified by Severity of Loes Score at Baseline

		Change from Baseline to Month 24									
						PedsQL					
				Weschler IQ			Psychosocial				
Parameter	Statistics	NFS	Loes	FSIQ	PrIQ	Physical	Social	School	Emotional	Overall	Total
Loes < 4.5	N	28	28	20	28	26	26	22	26	26	26
	Median	0.0	1.0	-5.0	-2.5	0.0	0.0	-2.5	0.0	-2.8	-2.7
	(25%, 75%)	(0.0, 0.0)	(0.8, 6.3)	(-9.5, 2.0)	(-11.0, 5.0)	(-11.6, 2.2)	(-25.0, 0.0)	(-20.0, 10.0)	(-25.0, 5.0)	(-15.0, 7.3)	(-8.8, 5.5)
	Min, Max	0, 1	0, 12	-32, 12	-36, 30	-63, 66	-60, 35	-35, 55	-60, 20	-47, 15	-45, 32
Loes ≥ 4.5	N	4	4	2	3	3	3	2	3	3	3
	Median	0.5	7.5	-22.0	-23.0	-25.0	-35.0	-15.0	-5.0	-16.7	-21.7
	(25%, 75%)	(0.0, 9.0)	(5.3, 12.0)	(-36.0, -8.0)	(-23.0, -8.0)	(-31.3, 0.0)	(-55.0, 0.0)	(-20.0, -10.0)	(-15.0, -5.0)	(-35.0, -11.7)	(-30.7, -7.6)
	Min, Max	0, 17	4, 16	-36, -8	-23, -8	-31, 0	-55, 0	-20, -10	-15, -5	-35, -12	-31, -8
Overall	N	32	32	22	31	29	29	24	29	29	29
	Median	0.0	2.8	-5.5	-6.0	0.0	0.0	-7.5	0.0	-4.7	-4.3
	(0.0, 0.0)	(1.0, 7.0)	(-11.0, 2.0)	(-13.0, 3.0)	(-12.5, 0.0)	(-30.0, 0.0)	(-20.0, 10.0)	(-15.0, 5.0)	(-15.5, 4.2)	(-10.8, 4.3)	
	Min, Max	0, 17	0, 16	-36, 12	-36, 30	-63, 66	-60, 35	-35, 55	-60, 20	-47, 15	-45, 32

Source: ALD-102 Table 44.2.10; Data cut date: 23 October 2020 (ALD-102)
Abbrev.: FSIQ, full scale IQ; NFS, neurologic function scale; PrIQ, performance IQ (PIQ, PRI or FRI)

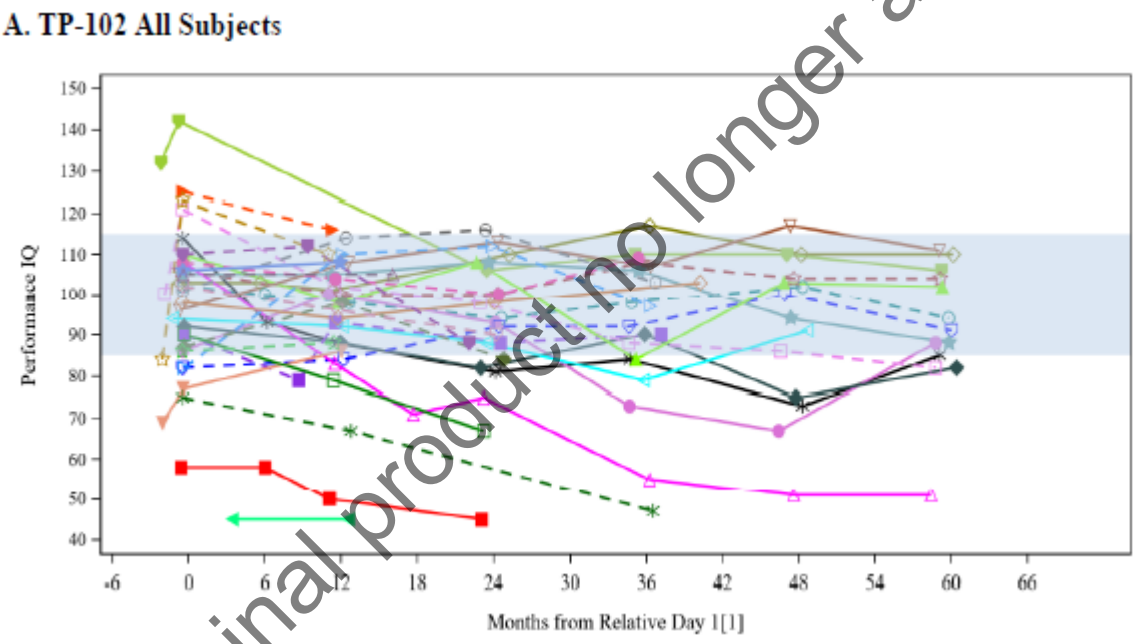
As anticipated from previous literature (Moser et al. 2000; Pierpont et al. 2017), subjects in TP102 with a Baseline Loes score of <4.5 had less evidence of disease progression at Month 24. Subjects with Baseline Loes scores of ≥4.5 to ≤ 9 (N=4) generally showed minimal disease progression as measured by NFS during this period, but did have larger changes in Loes score, WIQ and PedsQL (based on data collected in 2-3 subjects for WIQ and PedsQL). This was expected from reports in literature, but importantly the majority of these subjects still showed stabilisation of disease (see by-subject discussion in response to Question 78) suggesting eli-cel treatment was beneficial for subjects with Loes score ≤ 9.

Neuropsychological Tests

Subjects underwent a panel of neuropsychological tests, as appropriate for their age, including age appropriate Weschler IQ tests. These analyses were exploratory, and the consistency and quality of data collected was variable between subjects and between sites. Many study subjects were treated and had their IQ evaluated in a country where their native language was not spoken. The scores that are obtainable from these tests include performance IQ (PIQ), perceptual reasoning (PRI), fluid reasoning (FRI), processing speed index (PSI), working memory index (WMI), verbal IQ (VIQ)/ verbal comprehension index (VCI) and full scale IQ (FSIQ).

The PIQ/PRI/FRI was selected for focus due to this domain's relevance to the CALD patient population. For these analyses, PIQ, PRI and FRI are grouped together, as they assess similar domains and are the main assessment of performance IQ for their respective tests. The PIQ/PRI/FRI (hereafter referred to as performance IQ [PrIQ]) tests require actual physical handling of certain objects, such as picture cards and puzzles, in front of the examiner or psychologist. These assessments attempt to overcome biases caused by language, culture, and education which could play a role in interpreting VIQ tests for some of the international subjects assessed in the study.

Figure 7 Performance IQ (PIQ/PRI/FRI) Over Time by Subject



Supportive study:

Ongoing Study ALD-104

This is an ongoing open-label, multi-centre, single-arm, single dose 24-month study in paediatric patients with CALD receiving eli-cel After Myeloablative Conditioning Using Busulfan and Fludarabine.

The study started in 2019 and Data Cut for Interim CSR was on 21 February 2020. At the interim data cut for this analysis, 13 subjects had been treated with eli-cel. Enrolment is ongoing. Approximately 35 subjects are planned to be treated.

Differences in eligibility criteria and treatment compared to study ALD-102:

- The DP VCN specification lower limit was increased from 0.5 to 0.7 c/dg on 19 January 2017.
- The conditioning regimen in study ALD-104 consisted of busulfan and fludarabine used instead of bulsulfan and cyclophosphamide as in study ALD-102.

Study objectives

• To evaluate the efficacy and safety of eli-cel after myeloablative conditioning with busulfan and fludarabine in subjects with CALD

Study endpoints

The same as for study ALD-102, although for most endpoints, the month 24 evaluation timepoint has not yet been reached.

Fludarabine Dosing

Per ALD-104 Protocol V1.1 through ALD-104 Protocol V3.0, fludarabine dosing was as follows:

On Rel Days -8 and -7 (where Rel Day 1 is defined as day of eli-cel infusion), fludarabine IV (30 mg/m2) was to be given alone.

On Rel Days -6, -5, -4, and -3, fludarabine IV (30 mg/m2) was to be given one hour before the first daily infusion of busulfan IV.

With the introduction of ALD-104 Protocol V3.1 for study centres in the US and the UK, and ALD-104 Protocol V4.0 for all other sites, fludarabine dosing was adjusted such that fludarabine IV (40 mg/m2) was to be given on Rel Days -6, -5, -4, and -3, one hour before the first daily infusion of busulfan IV, per institutional standards.

Fludarabine dosing was reduced due to concerns of long-term immune reconstitution, following viral adverse events and in light of available literature related to fludarabine dosing in children (Harris et al. 2018; Langenhorst et al. 2019).

Statistical methods

The number of subjects planned to be infused with Lenti-D Drug Product was approximately 20 according to the SAP (the only version, dated 30 October 2019), but has been increased to 35 in a later amended protocol in order to accrue additional efficacy and safety information.

Analysis populations, the primary endpoint definition, and statistical methods in general, are similar to those described for the pivotal study ALD-102. The exception is that for study ALD-104 there was no benchmark comparison planned for the primary endpoint, and no separate cohorts were defined for interim and final analysis. The planned analysis included interim safety analyses to support regulatory submissions and a final analysis when all treated subjects complete the study.

Baseline data

Figure 8 Subject disposition

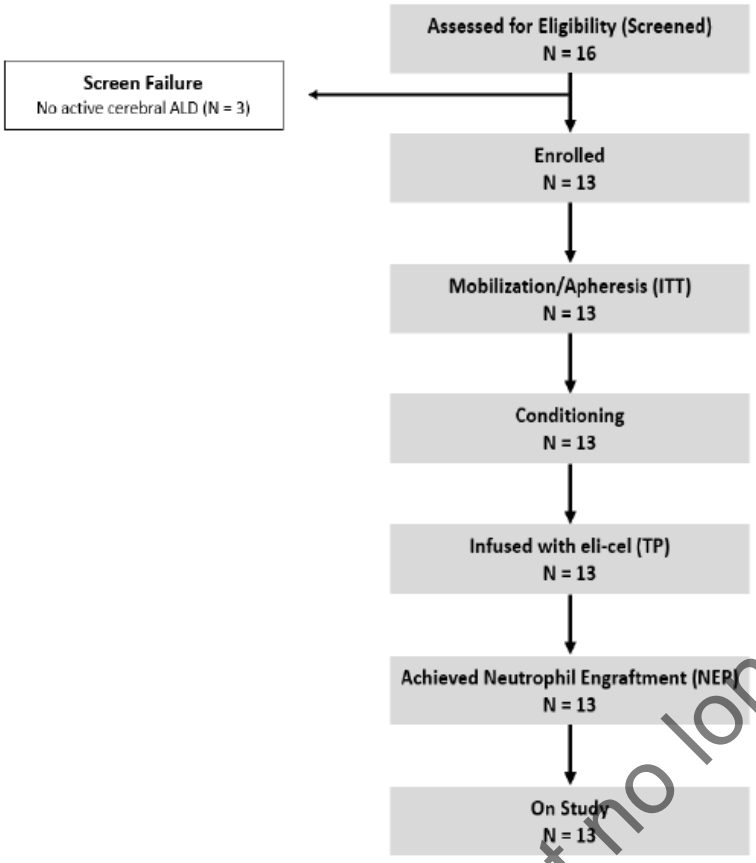


Table 10 Subject Demographics

Parameter	ITT (N = 13)
Age at ICF (years)	
Median	8.0
Min, Max	5, 12
Age at ICF category, (years) n (%)	
≥ 2 to < 6	
≥ 6 to < 12	
≥ 12 to < 18	
Age at diagnosis of CALD (years)	
Median	8.0
Min, Max	4, 10
Age at eli-cel infusion (years)	
Median	9.0
Min, Max	5, 13

Parameter	ITT (N = 13)
Age at ICF (years)	
Median	8.0
Min, Max	5, 12
Age at diagnosis of CALD (years)	
Median	8.0
Min, Max	4, 10
Age at eli-cel infusion (years)	
Median	9.0
Min, Max	5, 13
Gender, n (%)	
Male	13 (100)

Source: Table 14.1.3.1
Abbrev.: ICF, informed consent form; CALD, cerebral adrenoleukodystrophy.

Table 11 Baseline Characteristics (ITT)

Parameter	ITT (N = 13)
Method of diagnosis[a], n (%)	
MRI	13 (100)
Elevated VLCFA	11 (84.6)
ABCD1 Genotyping	9 (69.2)
Family History	5 (38.5)
Diagnosed primary adrenal insufficiency	5 (38.5)
Other	2 (15.4)
Signs and symptoms, n (%)	
Adrenal insufficiency	12 (92.3)
Asymptomatic	10 (76.9)
Hyperactivity	2 (15.4)
Seizures	1 (7.7)
Other	1 (7.7)

Baseline Neurologic Function Score	
0	12 (92.3)
1	1 (7.7)
Baseline Loes score	
Median	3.00
Min, Max	1.0, 7.0
Baseline Loes pattern, n (%)[b]	
Pattern 3 and/or 4 only	3 (23.1)
Patterns include 1, 2, 5[c]	9 (69.2)
Other	1 (7.7)
Time from informed consent to eli-cel infusion (days)	
Median	69.0
Min, Max	62, 79
Time from diagnosis of CALD to eli-cel Infusion (months)	
Median	4.93
Min, Max	3.2, 28.7

As of the interim data cut for this report, only 7 subjects have completed the Month 6 Visit and no subjects have completed the Month 24 Visit. Due to the limited time of follow-up, only preliminary data on select pharmacodynamic efficacy endpoints are presented in this interim report (please, refer to PD section for these results)**.**

During the approval procedure, efficacy and PD data for study ALD-104 from the data cut-off of 09 Oct 2020 have been provided. In total 19 subjects have received eli-cel, all subjects remain on study, no MFDs, no deaths, no rescue cell treatment nor rescue allo-HSCT have been performed. The median (min, max) duration of follow-up is 8.64 (0.1, 16.8) months. Only 8 subjects have completed their Month 12 Visits, and no subjects have reached their Month 18 Visit or beyond. Thus, no efficacy data for the primary endpoint MFD-free survival at month 24 are available from this data cut-off.

Supportive study:

Ongoing study LTF-304

A multi-center, non-interventional long-term safety and efficacy follow-up study for subjects with CALD who have been treated with eli-cel in the Sponsor's parent studies, ALD-102 and ALD-104. After completion of the parent study (including approximately 2 years of follow-up), subjects are consented to enroll in Study LTF-304. During Study LTF-304, subjects are followed every 6 months from 2 years through 5 years post-drug product infusion and then annually from 5 years through 15 years post-drug product infusion.

The study started in 2016 and the Data cut-off for interim analysis is 31 Jan 2020

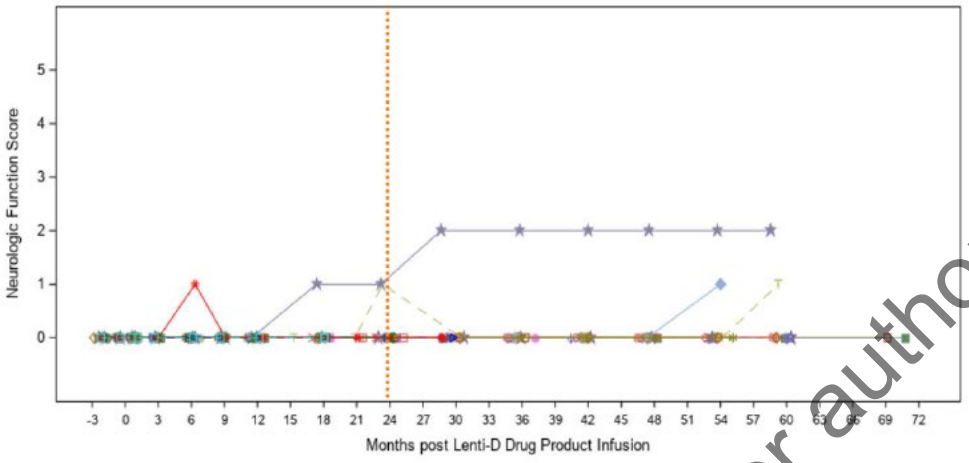
At the time of data cut-off, 21 patients from study ALD-102 had enrolled in the LTF-304 study, with a median (min, max) duration of follow-up of 56.44 (22.1, 70.7) months after drug product infusion.

Maintenance of efficacy-results:

- PD data are presented in the PD section
- MFD-free survival: 21/21 subjects maintaining their MFD-free status
- Overall Survival: 21/21 subjects alive as of their last follow-up on study

- GdE status: Four subjects had a change in GdE status at latest assessment compared with their Month 24 Visit: 3 subjects changed from GdE+ to GdE- and 1 subject changed from GdE- to GdE+.
- NFS:

Figure 9 NFS Over Time by Subject



As seen in the figure above, 3 out of 18 (16.7%) subjects who had assessments after Month 24 had an increase in NFS of ≤ 3 at latest assessment compared to parent study ALD-102 Baseline:

• One subject had an NFS of 0 from parent study ALD-102 Baseline until his Month 24 Visit, at which time he experienced episodes of **incontinence**, resulting in an NFS of 1. This had resolved by Month 30, and he maintained an NFS of 0 until his last recorded visit at Month 60, when his NFS increased to 1 due to nonfebrile **seizures**.

• One subject had an NFS of 0 from parent study ALD-102 Baseline through the Month 48 Visit. At Month 54 (last visit prior to data cut date), the subject experienced **vision impairment**, and his NFS increased to 1.

• One subject developed **vision impairment** starting at Month 18 (NFS of 1).This subject also experienced an SAE of **Seizure** (Listing 16.2.7.2) within 24 months of drug product infusion (Rel Day 722) but this event occurred after his Month 24 assessment for NFS (Rel Day 706) so did not contribute to his NFS score at Month 24. He continued to experience both symptoms through his last recorded visit before the interim database lock (Month 60; NFS of 2). His vision impairment and apparent seizure disorder were attributed to disease progression by the site PI (PI communication).

Loes score

15 subjects had assessments beyond Month 24, and 13 maintained a stable Loes score as of latest assessment in study.

Figure 10 Loes score Over Time by Subject (Study LTF-304)

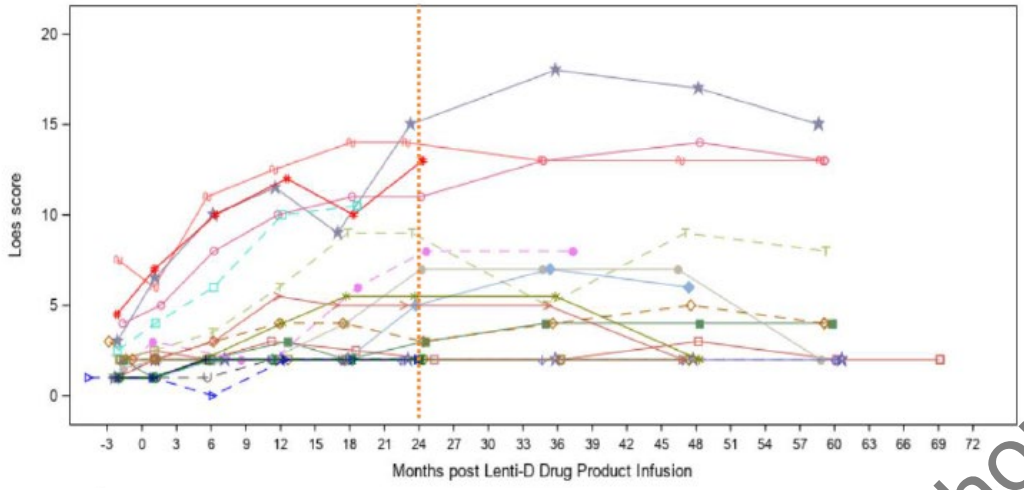


Table 12 Proportion of Subjects with Stable Loes Score

	Stable Loes Score[a]			
Parameter	**Month 24**	**Month 36**	**Month 48**	**Month 60**
N	20[b]	15	14	10
n (%)	16 (80.0)	13 (86.7)	12 (85.7)	8 (80.0)
Exact 95% CI	(56.3, 94.3)	(59.5, 98.3)	(57.2, 98.2)	(44.4, 97.5)

Source: Table 14.2.5.1

Note: Exact 95% confidence interval (CI) is obtained using the Clopper-Pearson method.

[a] Stable Loes score is defined as maintaining a Loes score ≤ 9 or not increasing by ≥ 6 points from Baseline

[b] As of the data cut for this interim CSR, 1 subject had enrolled into Study LTF-304 prior to completing the Month 24 Visit in parent study ALD-102. Therefore, only 20 subjects were evaluable at Month 24.

- **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 13 Summary of efficacy for trial ALD-102

Title: A Phase 2/3 Study of the Efficacy and Safety of Hematopoietic Stem Cells Transduced with Lenti-D Lentiviral Vector for the Treatment of Cerebral Adrenoleukodystrophy (CALD)		
Study identifier	IND Number: 15433 EudraCT Number: 2011-001953-10	
Design	Open-label, multi-center, single-arm, single dose study	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	24 months not applicable Separate extension study
Hypothesis	Descriptive	

Treatments groups	Initial cohort Transplant population (TP)		N=17
	Overall cohort transplant population (TP)		N=32
Endpoints and definitions	Primary endpoint	Month 24 MFD-free survival	Proportion of subjects who were alive, have none of the 6 MFDs at Month 24 Visit. MFDs were defined as: loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, complete loss of voluntary movement In addition; subjects should not have received rescue cell administration or allo-HSCT, not be withdrawn from study or lost to follow-up by month 24.
	Secondary endpoint:	Overall survival by month 24	Not needed
	Secondary endpoint	GdE- at month 24	Proportion of subjects who demonstrated resolution of gadolinium positivity on magnetic resonance imaging (MRI; i.e., GdE-) at Month 24.
	Secondary endpoint	Stable NFS at Month 24	Neurologic Function Score (NFS) is a 25-point scale that includes minor and major functional disabilities in ALD. Stable NFS is defined as maintaining an NFS $\leqslant$ 4 without an increase of > 3 from Baseline.
	Exploratory endpoint	Stable Loes score at Month 24	Loes score is a 34-point scoring scale of MRI abnormalities in CALD. Stable Loes score is defined as maintaining a Loes score $\leqslant$ 9 or not increasing by $\geqslant$ 6 points from Baseline.
Database lock	N/A, Interim analysis with Data cut-off 17 Jan 2020		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat (ITT)= Transplant population (TP)		
Descriptive statistics and estimate variability	Treatment group	Initial cohort	Overall cohort
	Number of subjects	17	32
	Month 24 MFD-free survival (%)	15/17 88.2%	20/23 87.0%
	95% confidence interval	63.6, 98.5	66.4, 97.2

	Overall survival by month 24 (%)	Not presented	95.5%
	95% confidence interval	Not presented	71.9, 99.3
	GdE- at Month 24 (%)	12/16 75%	17/21 81.0%
	95% confidence interval	47.6, 92.7	58.1, 94.6
	editorial nte - NFS at Month 24	15/16 93.8%	22/23 95.7%
	95% confidence interval	69.8, 99.8	78.1, 99.9
	Stable Loes score at Month 24	12/16 75%	16/21 76.2%
	95% confidence interval	47.6, 92.7	52.8, 91.8

Table 14 Inter-study comparison ALD-102 vs ALD-103 TPES MSD and NMSD

Title: Inter-study Comparison of main efficacy results between the pivotal study ALD-102 and concurrent control study with retrospective/prospective allo-HSCT ALD-103		
Design	ALD-102: Open-label, multi-centre, single-arm, single dose study LD-103: Retrospective and prospective single-arm comparator study	
	ALD-102 Duration of main phase: ALD-104 Duration of follow-up:	24 months, separate follow-up study LTF-304 48 months
Hypothesis	Descriptive	
ALD-102 ALD-103	Overall cohort transplant population (TP) TPES MSD population TPES NMSD population	N=32 N=10 N=17
Database lock	ALD-102: N/A, Interim analysis with Data cut-off 17 Jan 2020	

	ALD-104: Date of CSR: 15 Jul 2020

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	ALD-102: Intent to treat (ITT)= Transplant population (TP) ALD-103: TPES = strictly ALD-102-eligible transplant population MSD= matched sibling donor, NMSD= Not a matched sibling donor			
Descriptive statistics and estimate variability	Treatment group	**ALD-102** Overall cohort	**ALD-103** TPES MSD cohort	**ALD-103** TPES NMSD cohort
	Number of subjects	32	10	17
	Month 24 MFD-free survival (%)	N=23 20/23 87.0%	N=9 8/9 88.9%	N=9 6/9 66.7%
	95% confidence interval	66.4, 97.2	51.8, 99.7	29.9, 92.5
	Overall survival by month 24 (%)	95.5%	N=10 88.9%	N=17 86.3%
	95% confidence interval	71.9, 99.3	43.3, 98.4	54.7, 96.5
	GdE- at Month 24 (%)	N=21 17/21 81.0%	N=13 13/13 100%	
	95% confidence interval	58.1, 94.6	75.3, 100.0	
	*Stable NFS at Month 24	22/23 95.7%	N=12 12/12 100%	
	95% confidence interval	78.1, 99.9	73.5, 100.0	
	¤Stable Loes score at Month 24	16/21 76.2%	N=13 12/13 92.3%	

	95% confidence interval	52.8, 91.8	64.0, 99.8

Historical control studies

Study ALD-103

A Prospective and Retrospective Data Collection Study to Evaluate Outcomes in Males ≤17 Years of Age Undergoing Allogeneic Hematopoietic Stem Cell Transplantation for the Treatment of Cerebral Adrenoleukodystrophy

The study started in 2015 and Last Subject Last Visit for Clinical Study Report was 06 December 2019

Study conducted at multiple study centres: USA (5 sites), Germany (2 sites), UK (2 sites), Netherlands (2 sites), France (1 site), Italy (1 site), Spain (1 site), Argentina (1 site)

Key eligibility criteria were male and ≤ 17 years of age and a confirmed diagnosis of CALD as defined by abnormal VLCFA profile and cerebral lesion on brain MRI.

The following populations were evaluated for outcomes of allo-HSCT:

• The Intent-to-treat (ITT) population: all subjects who initiated conditioning

• The Transplant Population (TP): all subjects who received allo-HSCT

Additionally, data were summarised for all allo-HSCT subjects who had Baseline characteristics similar to those of subjects treated in Study ALD-102. These populations are defined as follows:

• Strictly ALD-102-Eligible Transplant Population (**TPES**), which includes subjects who received an allo-HSC infusion and satisfied the following criteria:

- NFS ≤1 at Baseline
- 0.5 ≤ Loes score ≤9 at Baseline
- Gadolinium enhancement (GdE+) at Baseline

• ALD-102-Eligible Transplant Population (**TPE**), which includes subjects who received an allo-HSC infusion and satisfied the following criteria:

- NFS ≤ 1 at Baseline
- Loes score ≤ 9 at Baseline
- Loes score ≥ 0.5 **or** GdE+ at Baseline

• GdE+ Transplant Population (**TPG**), which includes subjects who received an allo-HSC infusion and who were GdE+ at Baseline.

In addition, certain safety and efficacy endpoints were summarised for the following subgroups:

• Subgroups by donor type: subjects with a matched sibling donor (**MSD**), or without a matched sibling donor (**NMSD**)

Efficacy endpoints were basically the same as for studies ALD-102 and 104 above.

For efficacy results, please see Inter-study comparison below.

Study ALD-101 (retrospective untreated and allo-HSCT)

A Retrospective Study to Characterize the Natural History of Childhood Cerebral X-linked Adrenoleukodystrophy and to Investigate the Influence of Allogeneic Transplantation on Affected Subjects

Retrospective data collected between 2011 and 2012 and Date of CSR was 08 April 2015

Data collected from 5 study centers, 1 in France and 4 in the USA

Eligibility criteria:

Boys *3-15 years with confirmed diagnosis of CALD by either increased VLCFA levels or genetically confirmed mutations in the *ABCD1* gene with a Loes score of > 0 and < 15 and under observation/follow-up for at least 2 years following CALD diagnosis (for untreated) or after allo-HSCT, or until subject death if sooner.

*Exemptions were granted for 4 subjects who were younger than 3 years of age at CALD diagnosis (1 untreated [age 2 years] and 3 allo-HSCT-treated [ages 1 to 2 years])

Table 15 Summary of Key Patient Characteristics

Parameter, Statistic	Untreated Cohort (N=72)	Allo-HSCT Cohort (N=65)
Age at CCALD diagnosis (years)		
Median	8.0	8.0
Minimum, Maximum	2, 15	1, 13
Duration of follow-up from CCALD diagnosis (months)		
Median	52.2	54.1
Minimum, maximum	0.2, 259.2	4.8, 125.3
Baseline Loes Score, n (%)		
0.5 to 9	39 (60.9%)	40 (69.0%)
> 9	25 (39.1%)	18 (31.0%)
Baseline NFS score, n (%)		
≤ 1	24 (47.1%)	42 (75.0%)
> 1	27 (52.9%)	14 (25.0%)
Age at allo-HSCT (years)		
Median	NA	8.0
Minimum, Maximum	NA	2, 18
Donor HLA Match, n (%)		
HLA Matched Related	NA	13 (20.0%)
HLA Mismatched Related	NA	5 (7.7%)
HLA Matched Unrelated	NA	13 (20.0%)
HLA Mismatched Unrelated	NA	32 (49.2%)
Missing	NA	2 (3.1%)

HSCT was performed shortly after CCALD diagnosis for the Allo-HSCT Cohort (median 4.3 months). In the Allo-HSCT cohort, only 13 (20%) subjects had a human leukocyte antigen (HLA)-matched, related donor; 12 of these were matched sibling donors. The source of HSC's was umbilical cord (48%), bone marrow (48%), granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood (3%), and unknown (1%).

Overall, 82% had a myeloablative conditioning regime and 19% had a reduced intensity conditioning. The most commonly administered conditioning agents for allo-HSCT included busulfan (51 subjects; 78%), cyclophosphamide (32 subjects; 49%), anti-thymocyte globulin (22 subjects; 34%), and alemtuzumab (17 subjects; 26%). Other conditioning agents were given in < 25% of subjects.

Comparisons of Survival Between Untreated Versus Allo-HSCT Subjects

There was a statistically significant association between treatment (Allo-HSCT yes/no) and Kaplan-Meier estimated 5-year overall survival rate from CCALD diagnosis for all subjects (78% versus 55%, p = 0.0119), as well as for the subsets of subjects with progressive disease at baseline (i.e., baseline NFS > 1: 89% versus 25%, p = 0.0333; baseline GdE+: 84% versus 36%, p = 0.0015). However, allo-HSCT was not associated with significant improvement in estimated 5-year overall survival rates for subjects with early disease, nor in estimated 2-year overall survival rates in any subset analyzed, perhaps reflecting advances in nursing and rehabilitation care that has improved the short-term survival of severely sick patients with advanced CCALD. Nevertheless, results did suggest that analysing GdE+ subjects may increase the significance of any effect because GdE+ status is associated with rapidly progressive disease without treatment.

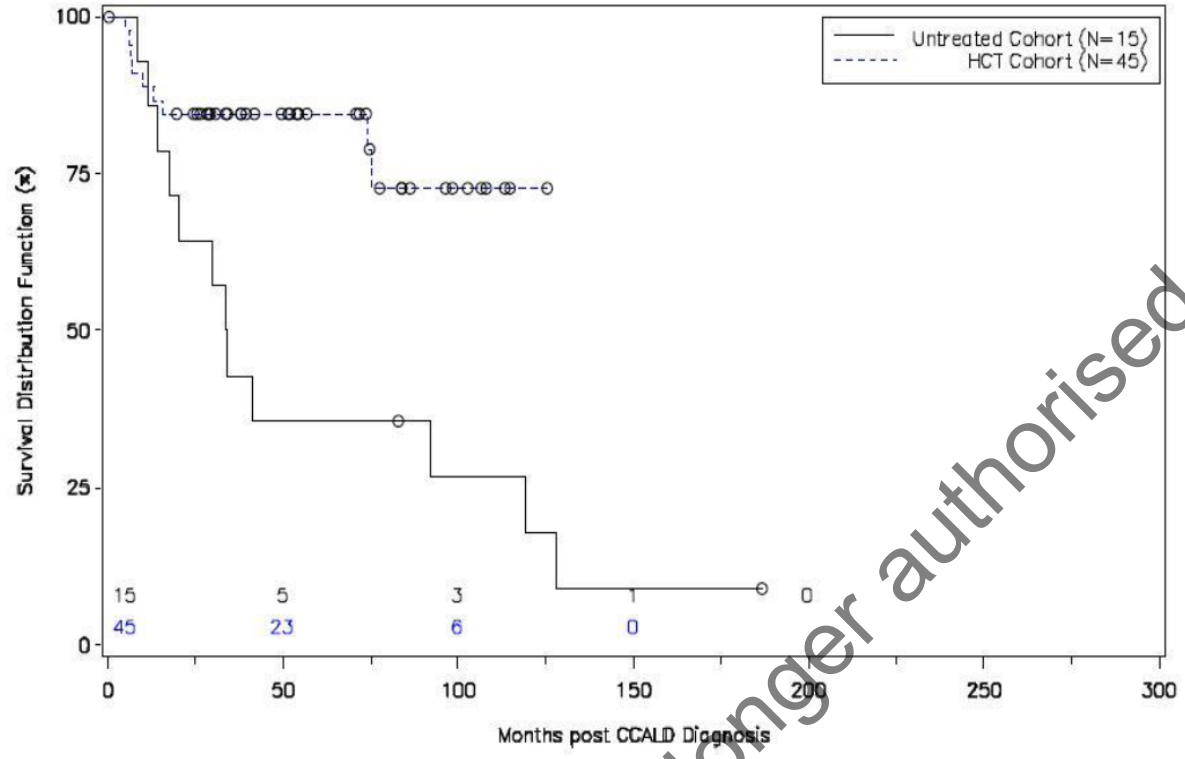
Table 16 Comparison of Kaplan-Meier Survival Estimates Between Untreated and HSCT Cohorts

Subgroup	To	Kaplan-Meier Estimated Survival Rate, % (N)		p-value
		Untreated cohort	Allo-HSCT cohort	
Overall survival, from CCALD diagnosis				
all	5-year	55% (N=72) [a]	78% (N=65) [a]	0.0119
baseline GdE+	5-year	36% (N=15) [a]	84% (N=45) [b]	0.0015
baseline NFS > 1	5-year	25% (N=27) [a]	89% (N=9) [a]	0.0333
baseline NFS ≤ 1	5-year	74% (N=24) [a]	79% (N=24) [a]	0.9917
baseline early disease	5-year	77% (N=18) [a]	94% (N=34) [b]	0.1134
MFD-free survival, from CCALD diagnosis, or from first GdE+ scan#				
baseline early disease	2-year	88% (N=18) [a]	91% (N=34) [b]	0.8002
baseline early disease AND GdE+	2-year		GdE+ before HSCT 88% (N=27) [b] 85% (N=27) [b]#	Sample size too small for untreated cohort
All GdE+		GdE+ at any time 29% (N=21) 29% (N=21) #		

[a] baseline is day of CCALD diagnosis
[b] baseline is last evaluation before HCT infusion
[c] early disease defined as baseline NFS ≤ 1 & Loes 0.5 to 9
p-values ≤ 0.05 in red font

Kaplan-Meier analysis of MFD-free survival did not allow an earlier detection of efficacy of allo-HSCT treatment than did analysis of overall survival for all subjects with early disease at baseline (2-year MFD-free survival rates of 88% untreated versus 91% allo-HSCT treated, for subjects with baseline NFS ≤ 1 & Loes 0.5 to 9), presumably because many of these subjects had slowly progressing disease. However, it was anticipated that analysing the GdE+ subset of subjects with early disease would increase the significance of the analysis, as well as make it more clinically relevant, because allo-HSCT is currently standard of care for CCALD patients with early stage disease (NFS≤1 & Loes 0.5 to 9) and GdE+ status. Kaplan-Meier estimated 2-year MFD-free survival rates for subjects who fell into this category were 85% for allo-HSCT subjects (N=27, NFS ≤ 1, Loes between 0.5 and 9, and GdE+ before allo-HSCT) but it could not be meaningfully calculated in untreated group because of small sample size (N=1 GdE+ at CCALD diagnosis, N=3 GdE+ at any time). However, the Kaplan-Meier estimated 2-year MFD-free survival for all GdE+ untreated subjects regardless of baseline NFS and Loes scores was 29%. Therefore, because the majority of GdE+ untreated subjects will have rapidly progressive disease, it is likely untreated subjects, if evaluated with sufficient frequency to detect GdE+ whilst still showing early disease, would also have low 2-year MFD-free survival rates.

Figure 11 Overall Survival Rate From Time of CCALD Diagnosis: Baseline GdE+ Subjects (Untreated Cohort and HCT Cohort) With Number of Subjects at Risk



Untreated Cohort: Failed=12, Censored=3 and HCT Cohort: Failed=9, Censored=36.

Inter-study comparisons

ALD-102 vs ALD-103 and ALD 101

Statistical methods

Analysis populations

The Transplant Population (TP), i.e. subjects who receive Lenti-D Drug Product in Study ALD-102 or Study ALD-104, and subjects who receive allo-HSCT in Studies ALD-101 and ALD-103, is abbreviated as TP-102, TP-104, TP-101, and TP-103, respectively.

The Successful Neutrophil Engraftment Population (NEP) consists of subjects who achieved neutrophil engraftment defined as having 3 consecutive absolute neutrophil count (ANC) laboratory values of ≥ 0.5 × 109 cells/L (after initial post-infusion nadir) obtained on different days by 42 days post-infusion of HSC. The NEP was used to compare hospitalisation after a successful NE from either Lenti-D Drug Product or allo-HSCT, only if the NEP is different from the TP.

Three baseline parameters, NFS, Loes score, and gadolinium enhancement (GdE) status, are important prognostic factors for CALD disease progression in untreated patients. Therefore, efficacy and safety results in TP-102 and TP-104 were compared with populations in Studies ALD-101 and ALD-103 with "similar" baseline disease characteristics with respect to these three parameters.

Table 17 Analysis populations comparable to Subjects in ALD-102

Population	Description
Strictly ALD-102-Eligible Transplant Population (TPES)	Subjects in TP-101 and TP-103 who have the same baseline characteristics in terms of NFS, Loes score, and GdE status that would make them strictly eligible for Study ALD-102, as follows: • NFS ≤ 1 at Baseline • Loes score ≥ 0.5 to ≤ 9 at Baseline • GdE+ at Baseline The TPES in Studies ALD-101 and ALD-103 are abbreviated as TPES-101 and TPES-103, respectively.
ALD-102-Eligible Transplant Population (TPE)	Subjects in TP-101 and TP-103 who have similar but not identical baseline characteristics in terms of NFS, Loes score, and GdE status that would make them eligible for comparisons with Study ALD-102, as follows: • NFS ≤ 1 at Baseline • Loes score ≤ 9 at Baseline • Loes score ≥ 0.5 or GdE+ at Baseline The TPE is defined to be more inclusive compared to the TPES. The TPE in Studies ALD-101 and ALD-103 are abbreviated as TPE-101 and TPE-103, respectively.
GdE+ Transplant Population (TPG)	Subjects in TP-101 and TP-103 who are GdE+ at Baseline. The TPG in Studies ALD-101 and ALD-103 are abbreviated as TPG-101 and TPG-103, respectively.
Strictly ALD-102-Eligible Untreated Population (UTES)	Untreated subjects from Study ALD-101 satisfying the following criteria: • GdE+ at some point during the study • NFS ≤ 1 at the time of the first GdE+ assessment during the study • Loes score ≥ 0.5 to ≤ 9 at the time of the first GdE+ assessment during the study. The timepoint of the first GdE+ assessment in UTES is identified as the Baseline visit for the inter-study analyses.
ALD-102-Eligible Untreated Population (UTE)	Untreated subjects from Study ALD-101 satisfying any of the following criteria: • GdE+ at some point during the study, and NFS ≤ 1 and Loes score ≤ 9 at the time of the first GdE+ assessment; or • No evidence of GdE+ throughout study (GdE- or GdE status unknown), and NFS ≤ 1 and Loes score ≥ 0.5 to ≤ 9 at the time of CALD diagnosis. The time of the first GdE+ assessment, or the time of CALD diagnosis for subjects with no evidence of GdE+, is identified as the Baseline visit for the inter-study analyses.
GdE+ Untreated (UTG) population	Untreated subjects from Study ALD-101 who are GdE+ at some point during the study. The time of the first GdE+ assessment in UTG is identified as the Baseline visit for the inter-study analyses.

Subgroups analyses were performed by donor category in subjects who received allo-HSCT. The categories included:
• Subjects who have a matched sibling donor (MSD)
• Subjects who do not have a matched sibling donor (NMSD).

Statistical analysis methods for the inter-study comparisons were primarily descriptive in nature. In addition to the efficacy success criterion based on the proportion of subjects who achieved Month 24 MFD-free survival in study ALD-102, a safety success criterion was defined for the inter-study comparisons.

The safety success criterion was based on achieving a statistically significant reduction in the proportion of subjects who experienced either acute GVHD (≥ Grade II) or chronic GVHD in TP-102 compared to TP-103 ($p<0.05$). Evaluable subjects for this analysis were those who received HSCI in Studies ALD-102 (TP-102) and ALD-103 (TP-103) who experienced either ≥ Grade II acute GVHD or chronic GVHD within 24 months post any HSCI or have been followed for at least 12 months after the latest HSCI. Subjects who experienced either ≥ Grade II acute GVHD or chronic GVHD within 24 months post any HSCI were considered failures for this endpoint. The Fisher's exact test was used for comparisons of the proportions. For all other comparisons, p-values were provided as descriptive measures. As one of the sensitivity analyses, the time to the first acute GVHD (≥ Grade II) or chronic GVHD was compared between TP-102 and the other analysis populations using competing risk analysis with engraftment failure (primary and secondary), transplant related mortality and death from other causes as competing risks. The cause-specific hazard model was used for the analysis.

The number and percentage of subjects who achieved Month 24 MFD-free survival as well as the two-sided exact 95% CI was presented. P-values for comparisons with TP-102 were obtained using Fisher's exact test. To be considered a success for Month 24 MFD-free survival, a subject must meet all following criteria:

1. Be alive 24 months post first HSCI.
2. Have not developed any of the MFDs by 24 months post first HSCI.
3. Have not received rescue cell administration or subsequent allo-HSCT by 24 months post first HSCI.
4. Have not withdrawn from the study (for reasons other than study termination) or lost to follow-up by 24 months post first HSCI.

This definition of MFD-free survival was different from the definition used in the CSR of Study ALD-101, where failure was defined as either having MFD or death by Month 24. Moreover, a wider window (-6 to +6 months) was allowed for Study ALD-101 to accommodate the unscheduled nature of data collection in this retrospective study, so that failures by Month 30 were included in this analysis for ALD-101.

MFD-free Survival was analysed using the Kaplan-Meier method. Death, MFD, and rescue cell administration or subsequent allo-HSCT were considered events; for all studies except ALD-101, subjects who did not experience any event were censored at their date of last contact (DLC). Due to the limited AE/SAE collection in ALD-101, subjects who did not experience any event in ALD-101 were censored at their last NFS assessment. The following results were to be presented:
- Kaplan-Meier estimates of the 25th, 50th (median) and 75th percentiles of event-free subjects with associated two-sided 95% CIs
- Number and percentage of events and censored observations
- Kaplan-Meier landmark estimates at 12, 24, 36, 48, 60, and 72 months post HSCI (Rel Days 365, 730, 1095, 1461, 1826, and 2191, respectively) with associated two-sided 95% CIs

- Restricted mean survival time (RMST) along with the standard errors at 24, 48, and 60 months post HSCI (Rel Days 730, 1461, and 1826 respectively)
- p-values based on the log rank test for comparison with TP-102
- Kaplan-Meier plots of the survival function.

Overall survival was analysed similarly to the analysis of the MFD-free survival.

NFS and Loes Score were analysed by descriptive statistics. GdE status over time was plotted by subject.

Demographics

For the ALD-102 study the median age at eli-cel treatment was 6.0 (4, 14) years and in the corresponding subpopulations (TPES) of the ALD-103 and ALD-101 studies, the median age was 8.0 (4,14) at first allo-HSCT.

Table 18 Baseline Disease Characteristics

	Eli-cel	Allo-HSCT							
	ALD-102	ALD-103				ALD-101			
Parameter	TP (N=32)	TPES (N=27)	TPE (N=35)	TPG (N=39)	TP (N=59)	TPES (N=26)	TPE (N=33)	TPG (N=45)	TP (N=65)
Time from CALD diagnosis to Rel Day 1 (months)[a]									
n	32	27	35	39	59	26	33	45	65
Mean (SD)	7.14 (5.069)	12.60 (21.984)	10.55 (19.615)	11.97 (20.103)	9.18 (16.769)	8.42 (7.466)	8.72 (7.071)	8.73 (12.266)	8.56 (10.919)
Median (25%, 75%)	5.83 (3.70, 8.54)	3.52 (1.97, 9.17)	3.55 (2.07, 7.03)	3.29 (2.07, 9.17)	3.52 (2.14, 6.90)	4.98 (2.73, 13.27)	6.90 (3.22, 13.27)	3.98 (2.37, 10.15)	4.27 (2.33, 12.52)
Min, Max	2.5, 26.8	0.6, 78.0	0.6, 78.0	0.6, 78.0	0.6, 78.0	0.6, 29.1	0.6, 29.1	0.6, 72.9	0.4, 72.9
Baseline neurologic function score (NFS), n (%)									
0	31 (96.9)	26 (96.3)	34 (97.1)	30 (76.9)	43 (72.9)	19 (73.1)	23 (69.7)	24 (53.3)	29 (44.6)
1	1 (3.1)	1 (3.7)	1 (2.9)	5 (12.8)	7 (11.9)	7 (26.9)	10 (30.3)	8 (17.8)	13 (20.0)
>1 to <=4	0	0	0	3 (7.7)	4 (6.8)	0	0	9 (20.0)	10 (15.4)
>4	0	0	0	0	1 (1.7)	0	0	2 (4.4)	4 (6.2)
Missing	0	0	0	1 (2.6)	4 (6.8)	0	0	2 (4.4)	9 (13.8)
Baseline Loes score									
n	32	27	35	39	56	26	33	44	57
Median (25%, 75%)	2.00 (1.00, 3.00)	3.00 (1.00, 6.50)	3.00 (1.00, 6.00)	5.50 (2.00, 10.00)	4.25 (1.00, 9.00)	4.50 (2.00, 6.00)	4.50 (2.00, 6.00)	6.50 (3.50, 10.75)	6.50 (4.00, 10.00)
Min. max.	1.0, 9.0	1.0, 9.0	1.0, 9.0	1.0, 18.5	0.0, 18.5	0.5, 9.0	0.5, 9.0	0.5, 15.0	0.5, 15.0
Subgroup by Baseline Loes pattern, n (%)[b]									
Pattern 3 and/or 4 only	3 (9.4)	2 (7.4)	4 (11.4)	2 (5.1)	5 (8.5)	0	1 (3.0)	0	1 (1.5)
Patterns include 1, 2, 5[c]	29 (90.6)	24 (88.9)	30 (85.7)	36 (92.3)	44 (74.6)	18 (69.2)	22 (66.7)	36 (80.0)	43 (66.2)
Other	0	1 (3.7)	1 (2.9)	1 (2.6)	7 (11.9)	1 (3.8)	1(3.0)	1 (2.2)	1 (1.5)
Missing	0	0	0	0	3 (5.1)	7 (26.9)	9 (27.3)	8 (17.8)	20 (30.8)
Baseline GdE Status, n (%)									
GdE+	32 (100.0)	27 (100.0)	27 (77.1)	39 (100.0)	39 (66.1)	26 (100.0)	26 (78.8)	45 (100.0)	45 (69.2)
GdE-	0	0	8 (22.9)	0	13 (22.0)	0	5 (15.2)	0	7 (10.8)
Missing	0	0	0	0	7 (11.9)	0	2 (6.1)		13 (20.0)

Abbrev.:allo-HSCT, allogeneic hematopoietic stem cell transplantation; CALD, cerebral adrenoleukodystrophy; GdE, gadolinium enhancement; TP, Transplant Population; TPE, ALD-102 Eligible Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population; SD, standard deviation

[a] For subjects receiving HSC, Rel Day 1 is the day of first infusion; for untreated subjects in ALD-101, Rel Day 1 is the day of the first GdE+ for subjects with at least one GdE+ result, or the day of CALD diagnosis if no GdE+ result during the study.

[b] The five Loes patterns are 1=Parietal-occipital; 2=Frontal; 3=Pyramidal tracts involvement; 4=Cerebellar white matter involvement; 5=Combined parieto-occipital and frontal white matter involvement.

[c] This pattern includes any or all of 1, 2, 5. Subjects with this pattern may also have 3, 4 or other. The category 'Other' represents patterns that can't be described using one of the existing patterns (1 through 5).

Table 19 Month 24 MFD-free Survival (TP-102, TPES-103, TPES-101, TP-103)

	Eli-cel	Allo-HSCT			
	TP-102 N=32	TPES-103 N=27	TPES-101 N=26	Pooled TPES-103/101 N=53	TP-103 N=59
Number of subjects evaluable[a]	23	18	20	38	44
Month 24 MFD-free survival					
n (%)	20 (87.0)	14 (77.8)	16 (80.0)	30 (78.9)	28 (63.6)
Exact 95% CI	66.4, 97.2	52.4, 93.6	56.3, 94.3	62.7, 90.4	47.8, 77.6
Initial Failure of MFD-free Survival by Month 24, n (%)					
Death	0	1 (5.6)	1 (5.0)	2 (5.3)	7 (15.9)
MFD	1 (4.3)	0	1 (5.0)	1 (2.6)	4 (9.1)
Second HSCT	2 (8.7)	3 (16.7)	2 (10.0)	5 (13.2)	5 (11.4)
Withdrawal or lost to follow-up	0	0	0	0	0
Month 24 MFD-free survival, sensitivity analysis[b]					
n (%)	22 (95.7)	17 (94.4)	18 (90.0)	35 (92.1)	33 (75.0)
Exact 95% CI	78.1, 99.9	72.7, 99.9	68.3, 98.8	78.6, 98.3	59.7, 86.8

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CI, confidence interval; MFD, major functional disability; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population

Note: Month 24 MFD-Free survival criteria defined as: alive 24 months post first HSCT; have not developed any of the MFDs by 24 months post first HSCT; have not received rescue cell administration or subsequent allo-HSCT by 24 months post first HSCT; and have not withdrawn from the study (for reasons other than study termination) or lost to follow-up by 24 months post first HSCT..

Note: A subject will only be counted once under "initial failure"; while under "overall failures" a subject can belong to as many categories as apply.

[a] Evaluable subjects are defined for each study (Study ALD-101, ALD-102 and ALD-103) and described in the statistical analysis plan.

[b] As a sensitivity analysis, only first failures that are death or MFD are included.

Table 20 Month 24 MFD-free Survival: Comparison by Donor Subgroup (TP, TPES)

	Eli-cel	Allo-HSCT							
	TP-102	TPES-103		TPES-101		Pooled TPES-101/103		TP-103	
	N=32	MSD N=10	NMSD N=17	MSD N=5	NMSD N=21	MSD N=15	NMSD N=38	MSD N=11	NMSD N=48
Number of subjects evaluable[a]	23	9	9	3	17	12	26	9	35
Month 24 MFD-free survival									
n (%)	20 (87.0)	8 (88.9)	6 (66.7)	3 (100.0)	13 (76.5)	11 (91.7)	19 (73.1)	8 (88.9)	20 (57.1)
Exact 95% CI	66.4, 97.2	51.8, 99.7	29.9, 92.5	29.2, 100.0	50.1, 93.2	61.5, 99.8	52.2, 88.4	51.8, 99.7	39.4, 73.7
Initial Failure of MFD-free Survival by Month 24, n (%)									
Death	0	1 (11.1)	0	0	1 (5.9)	1 (8.3)	1 (3.8)	1 (11.1)	6 (17.1)
MFD	1 (4.3)	0	0	0	1 (5.9)	0	1 (3.8)	0	4 (11.4)
Second HSCT	2 (8.7)	0	3 (33.3)	0	2 (11.8)	0	5 (19.2)	0	5 (14.3)
Withdrawal or lost to follow-up	0	0	0	0	0	0	0	0	0

Abbrev.: allo-HSCT, allogeneic haematopoietic stem cell transplantation; CI, confidence interval; MFD, major functional disability; MSD, matched sibling donor; NMSD, not a matched sibling donor; TP, transplant population; TPES, Study ALD-102 Eligible Transplant Population

[a] Evaluable subjects are defined for each study (Study ALD-101, ALD-102 and ALD-103) and described in the statistical analysis plan

Kaplan-Meier Estimated MFD-free Survival: (TP-102, TPES-103)

MFD-free survival was analyzed by Kaplan Meier analyses for several subpopulations. Comparisons of the Kaplan Meier analysis for TP-102 to TPES-103 are presented in Table and Figure below. MFD-free survival was also analyzed by allo-HSCT donor category and compared to TP-102.

The estimated MFD-free survival rate 36 months after allo-HSCT for TPES-103 was 63.2% (95% CI: 38.2, 80.4) compared to 90.3% (95% CI: 72.9, 96.8) for TP-102. While subjects in TP-102 failed this endpoint due to MFD development (1/32 subjects, 3.1%) and second HSC infusion (2/32 subjects, 6.3%, due to Investigator decisions), subjects in the TPES-103 failed due to death (3/27 subjects, 11.1%: 1 due to GVHD, 1 due to cardiac arrest, and 1 due to septic shock) and second allo-HSCT (5/27 subjects, 18.5%, due to primary or secondary graft rejection). Thus, the main reasons for failure in the TPES-103 population are related to immuno-incompatibility between donor and recipient.

Notably, while the rate of MFD-free survival is stable over time by approximately 18 months after eli-cel infusion for TP-102, TPES-103 has a decrease in survival rate after this time point, indicating that while most events do occur in the first 18 months after allo-HSCT, there are still some events that occur at later time points. This is consistent with results reported in the literature for allo-HSCT.

Overall, the hazard ratio of 0.261 [95% CI: 0.069, 0.986]), suggests that eli-cel reduces the risk of failing the MFD-free survival endpoint by up to 73.9% compared to allo-HSCT treated subjects in TPES-103.

When TP-102 was compared to subgroups separated by donor type, it was observed that MFD-free survival rate 36 months after HSC infusion for the TP-102 showed a trend to be higher than TPES-103-

MSD subjects, and is higher than TPES-103-NMSD subjects (Table 11). Overall, based on observed hazard ratios of 0.519 (95%CI: 0.086, 3.124) and 0.187 (95% CI: 0.046, 0.757), TP-102 reduces the risk of failing the MFD-free survival endpoint by 48.1% and 81.3% compared to MSD and NMSD subjects in TPES-103, respectively.

These findings suggest that treatment with eli-cel confers improved MFD-free survival over time when compared to that of subjects receiving allo-HSCT who have the same Baseline disease characteristics. This difference is mainly due to events in the TPES subgroups associated with immuno-incompatibility between donor and recipient.

Table 21 Kaplan-Meier Estimated MFD-free Survival: (TP-102, TPES-103)

	Eli-cel	Allo-HSCT (TPES-103)		
	TP-102 (N = 32)	All (N = 27)	MSD (N = 10)	NMSD (N = 17)
MFD-free Survival (months)				
Median (95% CI)	- (-, -)	- (25.8, -)	- (23.0, -)	- (2.2, -)
25th Percentile (95% CI)	- (16.2, -)	25.8 (1.4, -)	33.1 (23.0, -)	6.5 (1.3, -)
75th Percentile (95% CI)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
Hazard Ratio (95% CI)[a]		0.261 (0.069, 0.986)	0.519 (0.086, 3.124)	0.187 (0.046, 0.757)
MFD-free Survival Rate 24 months after HSCT (95% CI)	90.3 (72.9, 96.8)	75.9 (53.4, 88.6)	88.9 (43.3, 98.4)	70.6 (43.1, 86.6)
MFD-free Survival Rate 36 months after HSCT (95% CI)	90.3 (72.9, 96.8)	63.2 (38.2, 80.4)	74.1 (28.9, 93.0)	58.8 (27.5, 80.4)
MFD-free Survival Rate 48 months after HSCT (95% CI)	90.3 (72.9, 96.8)	63.2 (38.2, 80.4)	74.1 (28.9, 93.0)	58.8 (27.5, 80.4)
MFD-free Survival Rate 60 months after HSCT (95% CI)	90.3 (72.9, 96.8)	- (-, -)	- (-, -)	- (-, -)
Events, n (%)	3 (9.4)	8 (29.6)	2 (20.0)	6 (35.3)
Death	0	3 (11.1)	2 (20.0)	1 (5.9)
MFD	1 (3.1)	0	0	0
Second HSC Infusion	2 (6.3)	5 (18.5)	0	5 (29.4)
Censoring, n (%)	29 (90.6)	19 (70.4)	8 (80.0)	11 (64.7)
Withdrawal or lost to follow-up	1 (3.1)	2 (7.4)	2 (20.0)	0
Completed Study	0	4 (14.8)	1 (10.0)	3 (17.6)
Study ongoing at data cut	28 (87.5)	0	0	0
Study termination by Sponsor	0	13 (48.1)	5 (50.0)	8 (47.1)

Source: Interstudy Table 2.1.1.1, Interstudy Table 2.1.1.1.1, Interstudy Table 2.1.1.1.2

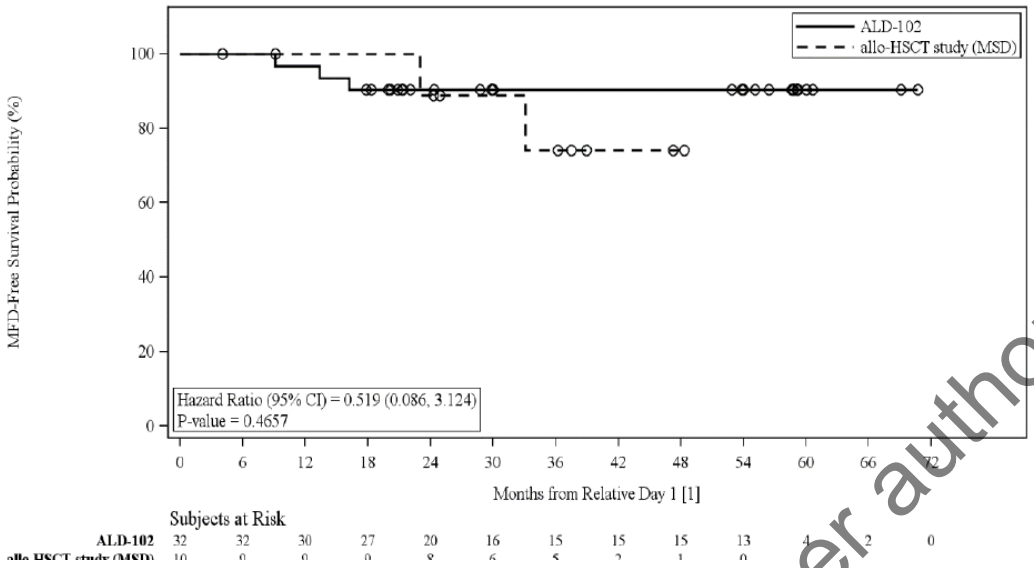
Abbrev.: CI, confidence interval; HSCT, Hematopoietic stem cell transplantation; MFD, major functional disability; MSD, matched sibling donor; NMSD, not a matched sibling donor; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population

Note: Estimates of MFD-free survival and restricted mean survival time are obtained using the Kaplan-Meier method, where events include deaths, MFDs, and rescue cell administration or second allo-HSCT. Subjects who did not experience any event are censored at their date of last contact.

[a] The hazard ratio of TP-102 vs. other analysis population is based on Cox regression model.

Figure 12 Kaplan Meier Analysis of MFD-Free Survival Over Time (TP-102, TPES-103)

A. TP-102 vs TPES-103-MSD population



B. TP-102 vs TPES-103-NMSD population

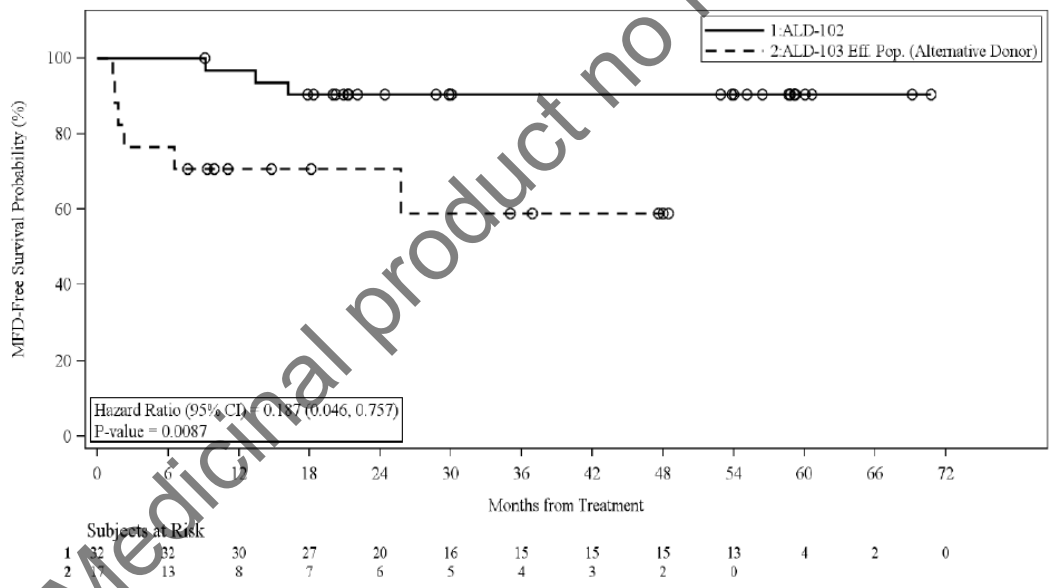


Table 22 NFS (TP-102, TPES-103, TPES-101, TP-103)

	TP-102 (N=32)	TPES-103 (N=27)	TPES-101 (N=26)	Pooled TPES-101/ 103 (N=53)	TP-103 (N=59)
Stable NFS at Month 24[a]					
Evaluable Subjects	23	12	11	23	26
n (%)	22 (95.7)	12 (100.0)	11 (100.0)	23 (100.0)	24 (92.3)
Exact 95% CI	78.1, 99.9	73.5, 100.0	71.5, 100.0	85.2, 100.0	74.9, 99.1
NFS at Month 24, n (%)					
n	23	12	11	23	26
0	20 (87.0)	11 (91.7)	9 (81.8)	20 (87.0)	17 (65.4)
1	2 (8.7)	1 (8.3)	0	1 (4.3)	6 (23.1)
>1 to ≤ 4	0	0	2 (18.2)	2 (8.7)	2 (7.7)
>4	1 (4.3)	0	0	0	1 (3.8)
Change from Baseline at Month 24, n (%)					
Decreased	0	0	0	0	1 (3.8)
No change	20 (87.0)	11 (91.7)	9 (81.8)	20 (87.0)	17 (65.4)
Increased ≤ 3	2 (8.7)	1 (8.3)	2 (18.2)	3 (13.0)	6 (23.1)
Increased > 3	1 (4.3)	0	0	0	2 (7.7)

Abbrev.: CI, confidence interval; NFS, neurologic function score; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population.

Note: The analysis is based on subjects who have non-missing Baseline and Month 24 assessments.

[a] Stable NFS at Month 24 is defined as maintaining a NFS <=4 without an increase of >3 points from Baseline.

Table 23 Loes Score (TP-102, TPES-103, TPES-101, TP-103)

	TP-102 (N=32)	TPES-103 (N=27)	TPES-101 (N=26)	Pooled TPES-101/103 (N=53)	TP-103 (N=59)
Stable Loes score at Month 24[a]					
Evaluable Subjects	21	13	17	30	26
n (%)	16 (76.2)	12 (92.3)	11 (64.7)	23 (76.7)	24 (92.3)
Exact 95% CI	52.8, 91.8	64.0, 99.8	38.3, 85.8	57.7, 90.1	(74.9, 99.1)
Loes score at Month 24					
n	21	13	17	30	26
Mean (SD)	6.50 (5.621)	4.50 (4.082)	7.15 (4.908)	6.00 (4.687)	4.90 (5.227)
Median (25%, 75%)	5.00 (2.00, 9.00)	2.00 (2.00, 6.00)	6.50 (3.00, 10.00)	5.00 (2.00, 9.00)	2.00 (1.00, 7.00)
Minimum, Maximum	2.0, 22.0	0.0, 15.0	2.0, 20.0	0.0, 20.0	0.0, 17.0
≤ 9, n (%)	16 (76.2)	12 (92.3)	11 (64.7)	23 (76.7)	21 (80.8)
> 9, n (%)	5 (23.8)	1 (7.7)	6 (35.3)	7 (23.3)	5 (19.2)
Change from Baseline, n (%)					
Decreased	0	4 (30.8)	0	4 (13.3)	6 (23.1)
No change	3 (14.3)	1 (7.7)	3 (17.6)	4 (13.3)	7 (26.9)
Increased < 6	11 (52.4)	7 (53.8)	12 (70.6)	19 (63.3)	11 (42.3)
Increased ≥ 6	7 (33.3)	1 (7.7)	2 (11.8)	3 (10.0)	2 (7.7)

Source: Interstudy Table 2.4.1, Table 2.4.4 and Amended ALD-103 Table 14.2.5.v2
Abbrev.: CI, confidence interval; SD, standard deviation; TP, Transplant Population; TPES, Strictly Eligible Transplant Population
Note: The analysis is based on subjects who have completed the Month 24 visit or would have reached the Month 24 visit if still in the study, at the time of data cut.
[a] Stable Loes score at Month 24 is defined as either maintaining a Loes score <=9 or not increasing a Loes score by >=6 points from Baseline.

Table 24 GdE Status at Month 24 (TP-102, TPES-103, TP-103)

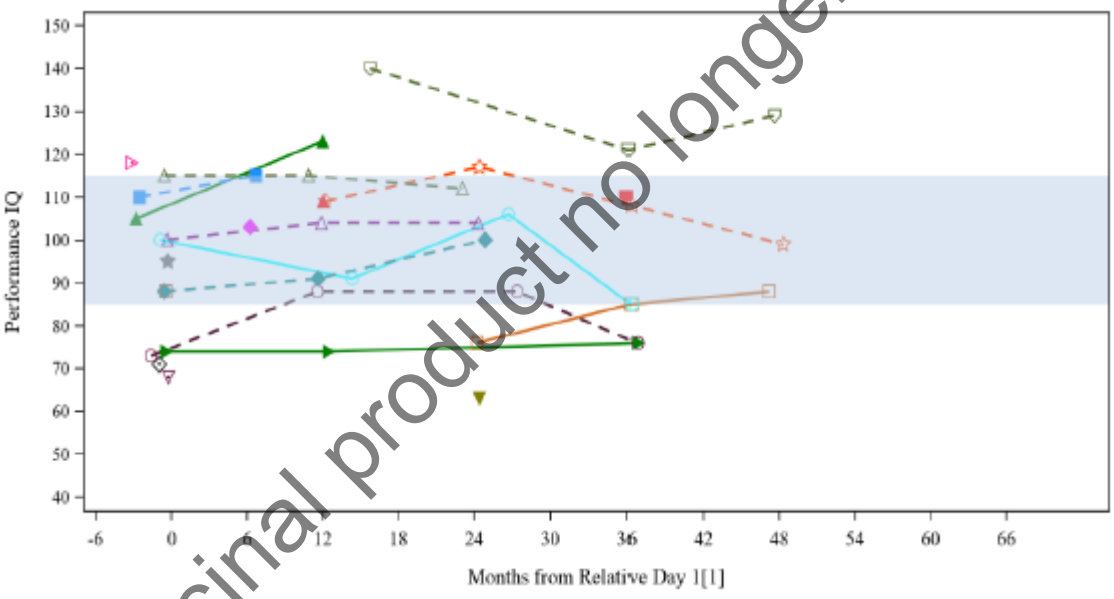
	TP-102 (N = 32)	TPES-103 (N=27)	TP-103 (N=59)
Subjects who are GdE- at Month 24			
Evaluable Subjects[a]	21	13	24
n (%)	17 (81.0)	13 (100)	24 (100)
Exact 95% CI	(58.1, 94.6)	(75.3, 100.0)	(85.8, 100.0)

Abbrev.: CI, confidence interval; GdE, contrast enhancement; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population

[a] Evaluable subjects are subjects who have completed the Month 24 Visit GdE assessment.

All subjects with the same Baseline disease characteristics (i.e., TPES-103) as TP-102 who had completed at least their Month 6 Visit became GdE- at some time after allo-HSCT. One subject in this population experienced re-emergence of GdE+ at their Month 12 Visit, but was reported to be GdE- at all other visits, thus 100% (exact 95% CI: 75.3, 100) TPES-103 subjects were GdE- at Month 24. This proportion of GdE- at Month 24 is higher than what was reported for TP-102 (81.0% [exact 95% CI: 58.1, 94.6]), but confidence intervals are overlapping.

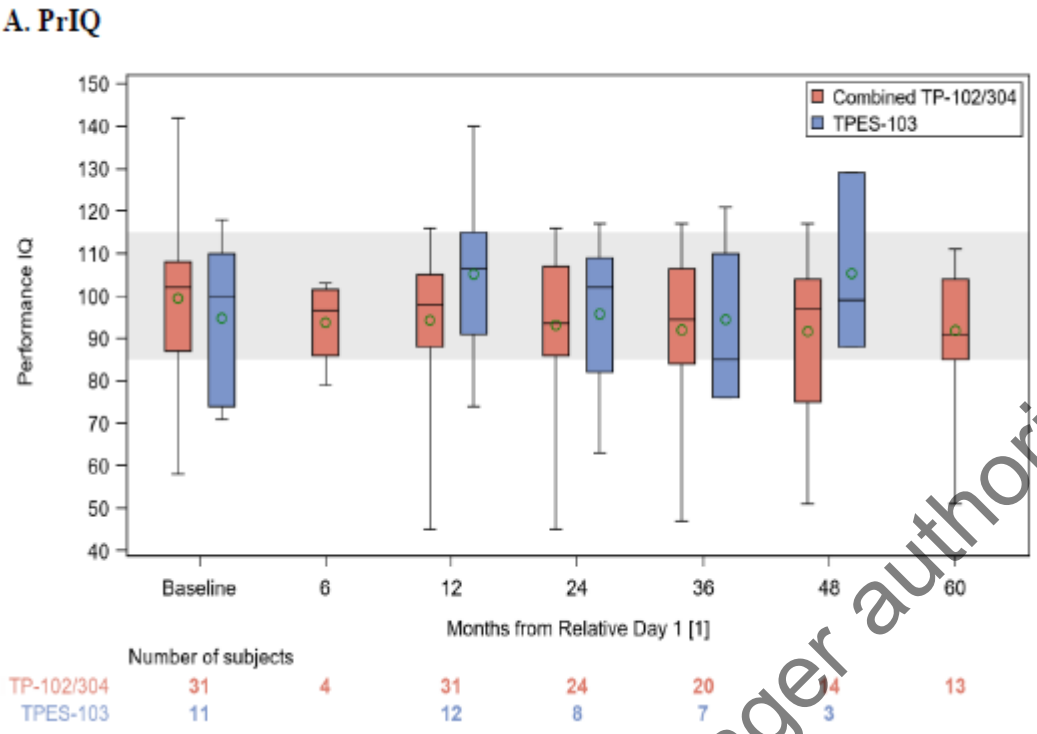
Figure 13 Performance IQ (PIQ/PRI/FRI) Over Time By Subject in TPES-103 (1st Allo-HSCT Period)



Note: Shaded are marks the normal range (ie, 100±15).
Note: Only assessments in the 1st allo-HSCT period are included for subjects receiving allo-HSCT.
[1] Relative Day 1 is day of eli-cel infusion for TP-102 and day of 1st allo-HSCT for TPES-103.

Performance IQ data for TPES-103 subjects are sparse, which hinders comparisons, but of the subjects with Baseline PrIQ and at least 1 additional assessment post allo-HSCT, most appear to have minimal changes in Performance IQ which are stabilised by Month 24.

Figure 14 Boxplots of Weschler IQ Eli-cel Treated TP-102/304 versus TPES-103



Source: Interstudy Figure 44.2.9.14.2.1, Figure 44.2.9.14.2.2; Data cut dates: 23 October 2020 (ALD-102), 02 November 2020 (LTF-304), 31 March 2020 (ALD-103)
Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population.
Note: Only assessments in the 1st allo-HSCT period are included for subjects receiving allo-HSCT.

Median Performance IQ in TP-102 stayed within the normal range with only a small decline over time, and the results appear similar between TP-102 and TPES-103. Generally, median values stayed near Baseline. There is more variability in the median Performance IQ over time for TPES-103, presumably due to the smaller number of subjects; the median Performance IQ dips below the normal range at Month 36, but otherwise also remains within the normal range.

2.5.3. Discussion on clinical efficacy

The indication applied for is: '*Skysona is indicated for the treatment of early cerebral adrenoleukodystrophy in patients less than 18 years of age, with an ABCD1 genetic mutation, and for whom a human leukocyte antigen (HLA)-matched sibling haematopoietic stem cell (HSC) donor is not available (see section 5.1).*'

The proposed indication is agreed upon.

It is concluded that although all patients in the clinical studies were males, a gender-neutral indication text is preferred to allow treatment also in rare cases of female childhood CALD patients. The SmPC includes the information in section 4.2 that female paediatric patients have not been included in the clinical studies.

In support of this indication, efficacy results from one pivotal study (ALD-102, N=32) and one ongoing long-term follow-up study (LTF-304) with subjects with CALD treated with eli-cel have been submitted. In addition, a second treatment study ALD-104 is ongoing but hadn't reached the cut-off timepoint for the primary efficacy endpoint at the time of approval.

As historical control groups, the retrospective study ALD-101 contains both untreated boys and boys treated with allo-HSCT and study ALD-103 is a contemporaneous study with boys treated with allo-HSCT. For long-term follow up of efficacy and safety, the ongoing LTF-304 study will contain all boys who were treated with eli-cel in the clinical studies. A planned registry will enrol boys treated with eli-cel post-approval or allo-HSCT.

Single-arm design of the studies with no comparator is an important limitation of the clinical development programme. Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is a therapeutic option that can stabilise progression of CALD, with the best outcomes observed if it is performed at the early stages of cerebral involvement. The severity of the disease, the rarity of the disease, the limitations of treatment options, the inability of transplant to be blinded, and the potential impact of time required to identify a donor match on cerebral disease progression precluded the conduct of a randomised controlled trial in the target patient population, this is acceptable and agreed during scientific advice given.

Dose-response

No dose-response studies have been performed, which is acceptable given the rarity and severity of the disease.

The applicant states that the minimum proposed dose of ≥ 5.0 x 10^6 CD34+ cells/kg eli-cel is the minimal dose that has been reported to lead to optimal neutrophil and platelet engraftment. Furthermore, there are no maximal recommended dose of CD34+ cells for infusion and no AEs related to high CD34+ cell doses have been reported in the literature. However, in line with Zynteglo, the Skysona SmPC 4.2 states that 'In clinical studies doses up to 38.2 × 10^6 CD34+ cells/kg have been administered'. This is supported.

Design and conduct of clinical studies

Pivotal study ALD-102

The main inclusion criteria were male 17 and younger with active CALD defined by elevated VLCFA values, Loes scores 0.5 to 9 on MRI, GdE+, NFS≤1. It is noted that a genetic diagnosis of ALD was not included in the inclusion criteria. However, the demographics data on individual subjects confirm that a genetic diagnosis based on detection of an *ABCD1* gene mutation had been performed for all included subjects. The genetic diagnosis is also included in the proposed Indication text, which is endorsed. The inclusion criteria related to early disease (Loes score, GdE+ and NFS) reflect the experience from allo-HSCT treatment of early CALD and are acknowledged.

Main exclusion criterion was availability of a willing 10/10 HLA-matched sibling donor. This exclusion criterion is reflected in the last sentence of the proposed Indication: "…for whom a human leukocyte antigen (HLA)-matched sibling haematopoietic stem cell (HSC) donor is not available".

The applicant states that the conditioning regimen used in this study, busulfan and cyclophosphamide, is a standard regimen used in allo-HSCT for CALD to achieve myeloablation and immunosuppression. The conditioning regimen is described in SmPC section 5.1.

The success criterion for study ALD-102 comparison to a clinically meaningful benchmark, has been accepted at scientific advice given. The efficacy results are also compared to the untreated cohort in ALD-101 and the allo-HSCT treated cohorts of studies ALD-101 and ALD-103.

The major endpoints MFD-free survival, NFS, Loes score have been specifically developed for CALD studies to represent outcomes that are clinically relevant in this condition. The selection of endpoints is in accordance with published results from successful trials using allo-HSCT to treat CALD. The primary and secondary endpoints were accepted in the EMA Central Scientific advice of 2018.

The study was not dimensioned on a formal power calculation, which is acceptable for a study in this limited patient population in a rare disease. Success criterion was defined based on a comparison of the primary efficacy endpoint (Month 24 MFD-free survival rate) to a clinically meaningful benchmark of 50%, which was agreed in the protocol assistance procedure.

The sample size was increased 3 times during the study. The applicant explains the amended sample size was needed in order to obtain study data on the investigational product from European manufacturing sites. Listing 16.1.6 in the submission for the study ALD-102 shows subjects receiving the investigational product from 3 specific manufacturing sites.

Statistical methodology applied was appropriate given the simple study design. Analysing only observed cases of success in Month-24 MFD-free survival, with no received rescue cell administration or allo-HSCT, is endorsed. Analysis of the primary endpoint based on the TP population instead of the ITT is not supported in principle in this condition, however this is not of concern for the prespecified primary analysis as the TP and ITT population are the same. Presentation of the four MFD-free survival criteria is complemented by subject listing of individual major functional disabilities. Comparison of the primary endpoint with the clinically meaningful benchmark of 50% has previously been accepted by the CHMP.

It is acknowledged that the primary analysis is performed as prespecified, based on the initially planned sample size, although 17 subjects were enrolled in the study instead of 15 originally planned.

Selection of GVHD at Month 24 as the primary safety endpoint is redundant due to necessity of a holistic approach in evaluation of the safety data. In addition to the analysis of proportions, time to GVHD was analysed in the inter-study comparisons.

Changes from the analysis planned in the study protocol were presented in the SAP but not changes between the SAP versions. There is no indication that the changes in the planned analysis were not data driven. The limitations of the study design pertained to the small sample size, no concurrent comparator arm, and selection of the primary efficacy endpoint designed for the study (i.e. with no adequate prior validation). Several of the methodological limitations are associated with general difficulties to design and conduct studies in rare patient populations with few or inadequate treatment options as is the case with CALD. In the primary efficacy analysis, subjects who discontinued prior to the Month 24 visit were considered treatment failures; however, the secondary endpoints were analysed based on non-missing values which violates the ITT principle and may bias the results. The impact of missing data on the study results was investigated in sensitivity analyses that showed similar trends as seen in the initial analysis performed on non-missing observations (see section on interstudy comparisons).

In study ALD-102, the issue of multiplicity has been avoided by limiting the primary analysis to the initial cohort instead of including the entire study population, which may be acceptable given the results for the overall cohort, and the confidence interval approach of the analysis (i.e. comparison to a fixed benchmark). However, no multiplicity adjustments were made in the inter-study comparisons which allows the interpretation of the results for descriptive purpose only. An implication is that the selection of the endpoints presented in the SmPC section 5.1 has not been based on statistical grounds but is driven by the obtained results and their clinical relevance.

Baseline

Median age at enrolment into study was 6 years and age span was 3-13 years in the transplant population. The majority of patients were 2-12 years and only 1 subject in each cohort was 13 years old at baseline. The age span at eli-cel infusion was 4-14.

Regarding the age span in the indication; there is experience with younger children (0-3 years) undergoing apheresis and conditioning from autologous and allogenous HSCT. Experience from allo-HSCT of CALD support the extrapolation of data to patients aged less than 3 years. No dose adjustment is needed for patients aged less than 3 years. The applicant has clarified that since CALD is detected based on radiologic signs of demyelination and normally, the myelination in the brain is not complete until the age of 12 months at the earliest, this is the earliest timepoint for a screening brain MRI as part of the diagnostic workup for CALD. NFS may be assessed from the age of 18 months. The Committee considers that the diagnosis of CALD will provide a limit of the lower age at treatment and no lower age limit is therefore needed in the Indication text.

Regarding the extrapolation from males to females; the Committee found evidence of very rare cases of female patients with cerebral symptoms of ALD with onset during childhood (Haas et al: Neonatal-onset adrenoleukodystrophy in a girl, Neonatal-onset adrenoleukodystrophy in a girl, Ann Neurology, 1982;12(5):449-457, and Lourenco et al, X-linked adrenoleukodystrophy in heterozygous female patients: women are not just carriers, Arq Neuropsiquiatr 2012;70(7):487-491. Thus, CAT considers that the treatment with Skysona should not be restricted to males and that female childhood CALD patients should be included in the indication text.

At baseline, only 1 of 32 patients had NFS 1, and 31/32 had NFS 0. The median Loes score at baseline was 2 with a range of 1-9. The median time from informed consent to eli-cel infusion was 67 days (span 58-89 days).

It is acknowledged that the ITT, TP and NEP populations are identical, and that the efficacy analysis therefore follows the ITT approach.

Efficacy results

The success criterion for the initial cohort of 17 patients was met. 15/17 (88.2 %, 95% CI 63.6, 98.5) of treated subjects met the primary endpoint of Month 24 MFD-free survival. Since the lower bound of the confidence interval (63.6%) was above the threshold of 50%, the study efficacy success criterion was met. The overall cohort showed similar results with 20/23 or 87.0% (95% CI: 66.4, 97.2) of treated subjects meeting the primary endpoint of Month 24 MFD-free survival with the lower bound of CI being 66.4%. This indicates that treatment with eli-cel is better than no treatment.

The secondary endpoints MFD-free survival over time and overall survival (1 subject died at month 22) didn't add any important information to the results from the primary endpoint.

The secondary endpoint resolution of gadolinium contrast enhancement at month 24, as considered possibly reflective of resolution of neuroinflammation, revealed somewhat conflicting results. Sixteen of 21 (76.2%) subjects had sustained GdE- by Month 24 with the median time to first GdE- for those who achieved sustained GdE- was 45 (29-551) days. On the other hand, an additional 5 subjects displayed re-emergence of GdE+ at some MRIs between month 6 and 24. The clinical significance of re-emergence of GdE+ is not known, but may be related to repair of the blood-brain barrier or remyelination, as has been seen in other similar conditions, e.g. MS. There was no association between GdE status and clinical outcome in this study.

Twenty out of 23 (87%) of evaluable subjects had an NFS of 0 at month 24 and 2/23 had an NFS of 1 (episodes of incontinence, visual impairment). The subject who died from disease progression had an NFS of 17 at the last assessment.

Sixteen out of 21 subjects who were evaluable had a stable Loes score at month 24, defined as ≤9 or ≤6 points increase from baseline. 10/21 had a change of ≤1. Of all 32 subjects, 9/32 didn't have a stable Loes score at their last assessment before data cut-off. The few (n=4) subjects with a baseline Loes score ≥ 4.5 had a greater worsening of Loes score (+7.5, vs +1.0) and Performance IQ (-23 vs -

2.5) from baseline to month 24 than subjects with a baseline Loes score of <4.5 (n=28). Baseline Loes <4.5 seems to be predictor of better outcomes (month 24 MFD-free survival 93%, overall survival 100%, stable NFS 100%, no changes in NFS for 92%, stable Loes score 87%). This confirms the data from Pierpont et al. (2017) on similar differences for allo-HSCT treated CALD subjects. To inform the treating physicians on the less favourable outcome in patients with a higher baseline Loew score, the following sentence has been added to SmPC section 5.1: 'A small subgroup of patients who had higher Loes scores at baseline tended to have a less favourable outcome.'

Performance IQ, an exploratory endpoint, was chosen as the main IQ result, since all patients did not have English as their native language and the performance IQ test overcomes such difficulties better than other parts of the full IQ test. This strategy is accepted. Data are presented for Performance IQ for all subjects in ALD-102 as individual plots over time. It appears from these plots that most subjects remained within the normal IQ range of 100 ± 15 points (i.e. 85-115 points). This is in line with what is seen after allo-HSCT. A few subjects (n=2) had a baseline IQ below 70 and stayed below 70 after treatment. A few (n=2) had a baseline IQ of above 70 and decreased below 70 after treatment. A persistent decrease in IQ down to <70 (i.e. cut-off for intellectual disability) may be regarded as a significant change in cognitive function. The outcome data on IQ adds granularity to the primary endpoint MFD-free survival and supportive outcome measures such as NFS and Loes score by providing measures of cognitive function.

As expected, patients who had a Loes score above 9 at any timepoint deteriorated to a greater extent in their IQ during follow-up than those whose Loes stayed below 9. In comparison, subjects in TPES-103 didn't provide much longitudinal performance IQ data. Only 8 of 20 subjects provided IQ data for 24 months or longer. Among these few patients, performance IQ tended to be rather stable over time. Similar graphs as were observed for performance IQ were shown for full IQ. In addition, the applicant has compared the in-house IQ data with IQ data from Pierpont et al. (2017), and their conclusion is that a similar trend towards some decline in IQ after allo-HSCT is seen as seen after eli-cel treatment.

Furthermore, the applicant has provided data from the Vineland II Adaptive Behavior Composite (VABS), which assesses adaptive behaviour in 4 domains: Communication, ADL, socialisation, motor skills with a mean composite score of 100 ± 15 (standard deviation). The VABS is normally used to measure adaptive behaviour to support the diagnosis of intellectual and developmental disabilities, autism, and developmental delays. This test reflects everyday functioning and was recorded for up to month 24 in the ALD-102 study. No VABS scores were collected in study ALD-103. VABS scores were more stable over time than IQ scores and here it was more obvious that particularly the subjects with Loes >9 at any timepoint deteriorated in VABS during the 24 months follow up after gene therapy but not the subjects with Loes below 9 during follow-up. The fairly stable VABS results over time indicate that these children manage their everyday life without much signs of developmental delay or autistic behaviour.

The PedsQL with its subdomains physical, emotional, social, and school functioning are presented both for ALD-102/LTF-304 and TPES-103. However, it appears that these results show a great variability both between subjects and in individual subjects over time, which makes any interpretation difficult.

The applicant has performed correlation analyses between Loees score changes and neuropsychological data, and PK/PD parameters and neuropsychological data. Negative correlations was seen between Loes score change from baseline and the two neuropsychological scores Performance IQ change from baseline and (correlation coefficient=-0.55, P=0.0014) and PedsQL change from baseline (correlation coefficient=-0.53, P=0.0030), but not between Loes score change and Full IQ change. For pharmacokinetic/pharmacodynamic markers and neuropsychological parameters, no correlations were seen, neither between total cell dose, median peripheral blood (PB) VCN, nor median PB %ALDP+ cells and the neuropsychological parameters full IQ, performance IQ and PedsQL.

In addition, no correlation was seen between age at treatment and change from baseline of the three neuropsychological parameters.

There was no apparent difference between drug product dose and VCN, age and Month 6 PD data for the 7 subjects with a Loes score >9 during the study and the whole study population, in these 7 subjects, mean eli-cel dose was 11.0 x 10^6 CD34+ cells/kg, mean eli-cel VCN 0.94 c/dg, mean age 6.6 years, mean month 6 peripheral blood VCN 0.47 c/dg, mean peripheral blood ALDP+ 13.2%.

Pediatric Quality of Life inventory generally showed a slight decrease from baseline to month 24, mainly driven by psychosocial health and emotional functioning.

Taken together, the primary endpoint MFD-free survival is supported by secondary and exploratory endpoints reflective of radiological signs of neuroinflammation and demyelinisation (GdE and Loes) and neurological function (NFS). These results show that treatment with eli-cel resulted in a clinically relevant stabilisation of disease by month 24. It is also noted that a limited number (N=23) of subjects have contributed to the primary endpoint Month 24 timepoint MFD-free survival at the time of the data cut-off for the MAA. In their response to questions, the applicant has provided data from a later cut-off (Q4 2020) with N=30 subjects having reached the timepoint for the Month 24 primary endpoint. At this data cut-off, the proportion of (90%) MFD-free survival was 90% (27/30, 95% CI 73.5, 97.9).

The applicant plans to capture neurocognitive function and school/work-life performance in the planned registry study REG-502, when these have been assessed as part of the standard of care of these children. Since the planned REG-502 study is a non-interventional study, these assessments cannot be required per protocol.

Supportive study ALD-104

Study ALD-104 started in Jan 2019, has treated 13 subjects, of which 7 have reached month 6 at the data cut-off 21 Feb 2020. However, no efficacy results have been presented from this interim analysis, only PD data up to month 6, which are presented in the PD section.

It is noted that study ALD-104 used a different conditioning treatment than study ALD-102, i.e. fludrabine instead of cyclofosfamide in addition to busulfan. With respect to efficacy, the applicant has provided preliminary efficacy results for study ALD-104 from a later data cut-off (autumn 2020), showing among the 19 patients treated so far no deaths, no MFDs and neither any study withdrawals nor rescue cell administration or rescue treatment with allo-HSCT in early follow-up (0-15 months). The efficacy and safety data for the busulfan + fludarabine conditioning regimen are currently insufficient for a full B/R evaluation. However, it is agreed that the safety data generated for eli-cel in combination with this regimen should be reflected in the SmPC.

Long-term follow-up study LTF-304

From the ongoing long-term follow-up study LTF-304, efficacy data from the first 21 subjects enrolled showed that efficacy was generally maintained during the currently reported follow-up period of 56.4 (22.1, 70.7) months. In LTF-304, no subjects had developed MFDs or died. Three subjects had a resolution of their GdE+ whereas 1 subject developed GdE+, 3 subjects had increased their NFS by 1 point, Loes score was generally stable over time beyond Month 24. For IQ data, please see study ALD-102 above.

Concurrent historical control study ALD-103 with allo-HSCT

Study ALD-103 was a retrospective and prospective concurrently run study to collect data on subjects treated with allo-HSCT for CALD. From the overall study population, the TPES subgroup was defined, which had the same eligibility criteria as the pivotal study ALD-102. Conditioning with the regimen Busulfan and Cyclophosphamide regimen appeared somewhat inferior to the Busulfan and Fludarabine

regimen (month 24 MFD-free survival: 64.7% vs 72.7%). A baseline Loes score above 9 was a clear prospective marker of treatment failure vs ≤9 (month 24 MFD-free survival: 33.3% vs 70.6%).

Retrospective historical control study ALD-101: Untreated and allo-HSCT treated subjects

The retrospective ALD-101 study collected data between 2011 and 2012, mainly from American centres. According to the protocol eligibility criteria patients were to be 3-15 years of age, inclusive, have increased VLCFA levels, a confirmed *ABCD1* gene mutation or both and a Loes score of 0-15. They were to have a follow-up time for at least 2 years or until death, if earlier. The median age at baseline was the same for the untreated and allo-HSCT-treated cohorts, 8.0 years. More patients in the untreated cohort had baseline NFS of >1 (53% vs 25%) and slightly more had a Loes score of >9 (39% vs 31%).

Age span at CALD diagnosis was 2-15 in the untreated cohort, and 1-13 in the allo-HSCT cohort. Since CALD in uncommon cases has been diagnosed at the age of 1, and treatment should be started as early as possible after CALD diagnosis, a lower age cut-off appears non-appropriate. It should also be noted that the age at treatment was 2-18 in the allo-HSCT cohort.

The overall 5-year survival from CALD diagnosis was 55% (N=72) in the untreated cohort vs 78% (N=65) in the allo-HSCT-treated cohort (p=0.0119). The comparison of 5-year survival or 2-year MSD-free survival between the two cohorts was hampered by the fact that the inclusion criteria didn't restrict the patient population to those with NFS ≤1, Loes score 0.5 to ≤9 and GdE+. When all these criteria were applied to the untreated cohort, the resulting subgroup was too small to enable any meaningful comparison. However, in the untreated cohort, it is clear from the data that GdE+ at baseline was a negative factor for 5-year survival (36%, N=15 vs 84% N=45 in allo-HSCT cohort, p=0.0015) and GdE+ at any time was an equally negative factor for 2-year MSD-free survival (29%, N=21). Likewise, a more advanced disease, as shown by NFS>1 at baseline had a negative impact on 5-year overall survival of 25% (N=27) in the untreated cohort vs 89% (N=9) in the allo-HSCT cohort.

Interstudy comparison between studies ALD-102, 103 and 101

In the interstudy comparison between studies ALD-102, 103 and 101, the age at first HSCT treatment was similar and so were baseline disease characteristics between ALD-102 and the corresponding TPES subgroups of studies ALD-103 and ALD-101.

Regarding the possible recruitment bias in terms of overlapping sites between studies ALD-102 and ALD-103, the applicant has explained that there were 4 sites recruiting for both studies and 2 of them recruited for ALD-103 while ALD-102 recruitment was active. These could be explained by pre-planned procedures. In general, sites preferred enrolling for ALD-102 only as long as recruitment was active which could create a bias for more positive outcomes in ALD-102. However, these two sites did only recruit for 1 study at a time.

Regarding the primary endpoint Month 24 MFD-free survival, the result in study ALD-102 of 87.0% (95% CI 66.4, 97.2) was in line with that of matched sibling donors in the TPES-103 population (88.9% (95% CI 51.8, 99.7), whereas the non-matched sibling donor subpopulation in TPES-103 showed a numerically worse result of 66.7% (95% CI 22.9, 92.5).

However, in the completed studies ALD-103 and ALD-101, only 67 % (18 of 27) and 77% (20 of 26) of subjects, respectively, strictly matched to the ALD-102 population, were evaluable for Month 24 MFD survival. Similarly, numbers of subjects evaluable for the analysis of NFS, Loes score and GdE status, are in most cases, in all submitted studies, based on the non-missing observations. The applicant explained the low evaluability numbers for the Month 24 MFD-free survival by the prespecified study-specific definitions of the evaluable population. However, the study specific definitions were not following the ITT principle, which is the reason for requesting sensitivity analyses. The applicant

therefore considered the most conservative approach to sensitivity analysis by counting non-evaluable subjects in TP-102 as having a negative outcome, while missing data for TPES-101 and TPES-103 were imputed as a success for the selected primary and secondary efficacy endpoints. The sensitivity analysis using the most conservative imputation approach did not change the conclusions on the main analysis for these parameters performed on non-missing observations. However, it was seen that the effect estimates are sensitive and prone to bias with increasing rate of missing data and should be interpreted cautiously. Another (supplementary) analysis was performed using parameters at the last available assessment instead of imputing missing data. The interpretation of the supplementary analysis is however questionable due to varying follow-up.

In study ALD-102, MFD-free survival was stable over time after Month 24, whereas it was further decreased in subjects treated with allo-HSCT in study ALD-103, in which it stabilised only from Month 36. Thus, the Kaplan-Meier estimated Month 36 MFD-free survival was 90.3% (95% CI: 72.9, 96.8) in ALD-102/LTF-304 vs 74.1% (28.9, 93.0) for the TPES-103 MSD subgroup and 58.8% (27.5, 80.4) for the TPES-103 NMSD subgroup. For patients treated with eli-cel, the most relevant comparison is with the TPES-103 MSD subgroup when it comes to comparing with the currently overall best available treatment. The comparison with the TPES-103 NMSD subgroup demonstrates the currently available best treatment option for the subgroup (70%) of CALD patients with no available HLA-matched sibling donor, such as defined in the Indication for Skysona. The worse MFD-free survival at month 36 in the allo-HSCT subgroups were related to immuno-incompatibility between donor and recipient, i.e. GvHD and second allo-HSCT due to graft rejection, and not to development of MFDs, i.e. disease progression, in the subjects.

Similar results were shown for NFS, with stable NFS at Month 24 (defined as maintaining a NFS <=4 without an increase of >3 points from Baseline) being 95.7% (95% CI 78.1, 99.9) versus 100% (73.5, 100.0) and 100% (71.5, 100.0) for the ALD-102, 103 and 101 studies, respectively.

However, a lower proportion (76.2%, 95% CI 52.8, 91.8) of boys in the ALD-102 study showed stable Loes score at month 24 compared to the TPES-103 of 92.3% (95% CI 64.0, 99.8), whereas in the TPES-101 subgroup these results were even worse (64.7%, 95% CI 38.3, 85.8). In the pooled TPES 101/103 subgroup they were similar (76.7%, 95% CI 57.7, 90.1), indicative of no true difference between eli-cel and allo-HSCT-treated groups.

Similarly, fewer patients in ALD-102 were GdE- at month 24 (81.0%, 95% CI 58.1, 94.6) compared to TPES-103 (100%, 95% CI 75.3, 100.0) and TPES-101 (100%, 95% CI 85.8, 100.0). Comparisons between the individual studies also showed that more subjects were GdE+ over the months after eli-cel treatment than after treatment with allo-HSCT in ALD-103. These results may indicate a slightly inferior effect on neuroinflammation by eli-cel treatment compared to allo-HSCT. This from an efficacy perspective, supports the restriction in the indication to boys for whom an HLA-matched sibling donor is not available, as reflected in the indication, until longer-term data on maintenance of efficacy and cognitive function is available.

2.5.4. Conclusions on clinical efficacy

Treatment with eli-cel (Skysona) of 4-14 years old boys diagnosed with CALD has been shown to be superior with regards to MFD-free survival compared to no treatment in a retrospective untreated CALD historical cohort and similar in efficacy in comparison to allo-HSCT treated retrospective and contemporaneous cohorts, especially similar efficacy was shown for Skysona and those subjects treated with allo-HSCT from an HLA-matched sibling donor. In comparison to subjects who received allo-HSCT from a non-matched donor, which currently is the available treatment option for the around 70% of the CALD boys, the MFD-free survival for eli-cel appears slightly superior.

In addition, comprehensive data from neuropsychological test results including age-appropriate Wechsler IQ test results, Vineland II Adaptive Behavior Scale Score (VABS) and Pediatric quality of life inventory (PedsQL) are available, supporting a stabilisation of cognitive function in the majority of children. The data have been supplemented with data from a later cut-off for the three clinical studies, confirming the results from the initial submission data cut-off.

The efficacy data is therefore considered appropriate to conclude on a positive B/R balance for the sought indication. However, the additional data collected post authorisation in the ongoing Study LTF-304 will be of value since the maintenance of efficacy over longer time than 60-80 months is currently unknown.

The CAT considered the following measure necessary to inform further on the long-term efficacy of the product:

In order to evaluate the long-term efficacy and safety of Skysona in patients with cerebral adrenoleukodystrophy (CALD), the MAH should submit final results of Study LTF-304.

In order to further characterise the long-term safety and efficacy, the MAH should conduct, and submit the results of a prospective observational Registry Study of patients with CALD treated with Skysona or allogeneic haematopoietic stem cell transplantation (Stargazer; REG-502).

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

2.6. Clinical safety

2.6.1. Clinical safety

Three clinical studies contributed safety information on Skysona: Studies ALD-102 (ongoing, interim data cut: 17 January 2020), ALD-104 (ongoing, interim data cut: 21 February 2020), and the long-term follow-up Study LTF-304 (ongoing, interim data cut: 31 January 2020). See Table 'Overview of clinical studies' in Section 3.3.5.

The applicant provided updated data in response to the Day 102 LoQ (Studies ALD-102 (ongoing, data cut: 23 October 2020), ALD-104 (ongoing, data cut: 09 October 2020), and the long-term follow-up Study LTF-304 (ongoing, data cut: 02 November 2020). In addition, late-breaking safety information was provided up to 05 February 2021.

Subjects in both parent studies ALD-102 and ALD-104 were eligible to enrol in study LTF-304 upon the completion of the Month 24 Visit of their respective study and were to be followed for an additional 13 years under the separate follow-up protocol LTF-304. At the time of the interim analysis, 21 subjects from ALD-102 had enrolled in Study LFT-304 and no subjects from ALD-104. As of the current cut dates, 25 subjects from ALD-102 are enrolled.

Two studies using allo-HSCT provided contextual safety data; Study ALD-103 is a completed prospective and retrospective data collection study of allo-HSCT. Study ALD-101 is a completed retrospective non-interventional data collection study of subjects either untreated or treated with allo-HSCT. Adverse event comparisons to allo-HSCT were mainly made with ALD-103. The Transplant Population (TP) consisted of subjects who received a Skysona infusion or allo-HSCT in each study.

Patient exposure

The TP-population for the combined studies ALD-102 and 104 (TP-102/104) included 45 subjects who were enrolled and treated in Studies ALD-102 (32 subjects) and ALD-104 (13 subjects), respectively. Safety data on 6 additional ALD-104 subjects were provided at the Day 120 update.

All subjects treated with Skysona underwent myeloablation with busulfan and lymphodepletion with either cyclophosphamide (Study ALD-102) or fludarabine (Study ALD-104).

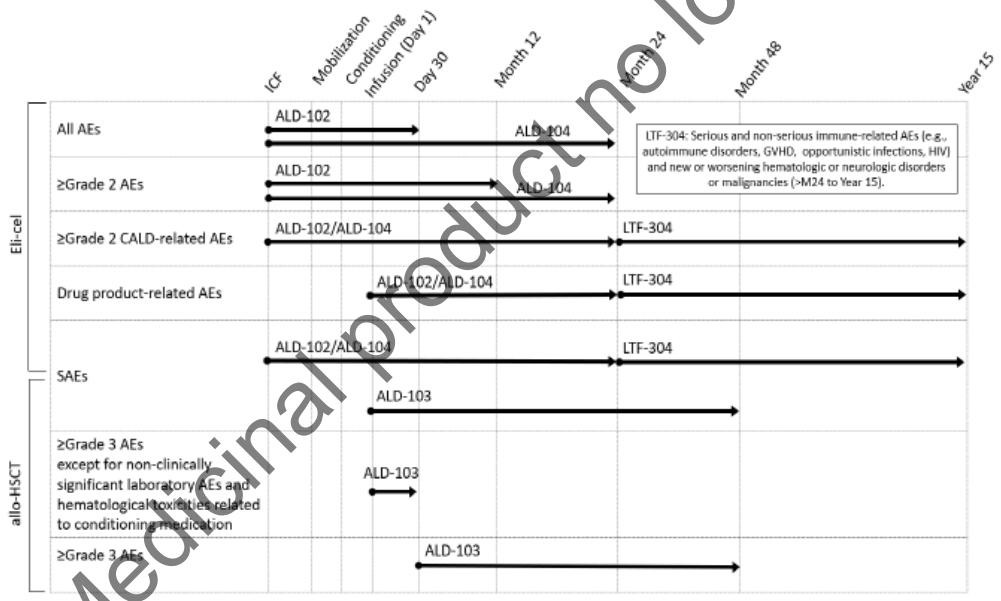
All treated subjects received a single lot of Skysona with a dose of ≥ 5.0 × 10^6 CD34+ cells/kg and met the minimum cell dose requirement per protocol.

Twenty-five subjects from Study ALD-102 continue to participate in the ongoing Study LTF 304 as of the 02 November 2020 data cut date; 1 subject discontinued after approximately 54 months of follow-up (subject declined further follow-up). The median total duration of follow-up of the ALD-102 and LFT-304 studies combined is 38.59 (13.4, 82.7) months.

In Study ALD-103, subjects were exposed to a variety of conditioning regimens according to institutional guidelines, including busulfan (96.6%) with either cyclophosphamide (47.5%) or fludarabine (64.4%) for lymphodepletion. In addition, some subjects received other conditioning agents such as anti-thymocyte globulin (47.5%) and/or alemtuzumab (23.7%). Nine of 59 (15.3%) subjects in Study ALD-103 required a second allo-HSCT; 1 of these subjects subsequently underwent a third allo-HSCT. Subjects in TP-103 had a median (min, max) follow-up time of 23.0 (0.9, 49.5) months.

Adverse events

The timing and strategy of AE collection for Studies ALD-102/ALD-104 and LTF-304, as well as for Study ALD-103, are shown in the figure below. Rel Day was defined such that "Rel Day 1 (or Day 1)" was the day of Skysona infusion.



Source: CSR ALD-102, CSR ALD-103, CSR ALD-104, and CSR LTF-304
Abbrev.: CALD, cerebral adrenoleukodystrophy; AEs, adverse events; ICF, informed consent form; SAEs, serious adverse events
Note: Data from Study LTF-304 are included with parent study (ALD-102) in the Inter-study Tables in this SCS.

Treatment-emergent AEs were defined as AEs starting during or after the first HSCI (i.e, Rel Day 1).

Safety comparisons to ALD-103 were limited to the following: ≥ Grade 3 AEs; ≥Grade 3 AEs related to HSCI; and SAEs. Non-clinically significant laboratory AEs and haematological toxicities related to conditioning medication for first 30 days after any allo-HSC infusion were not collected.

Neutrophil engraftment (NE) was defined as 3 consecutive absolute neutrophil counts (ANC) ≥ 0.5 × 10^9/L (after initial post-infusion nadir) obtained on different days by 42 days post-HSCT (Rel Day 43).

Platelet engraftment (PE) was defined as 3 consecutive platelet counts ≥ 20 × 10^9cells/L obtained on different days while no platelet transfusions were administered for 7 days immediately preceding and during the evaluation period.

No primary or secondary NE failure was observed in evaluable TP-102/104 subjects. Two subjects from Study ALD-104 were considered unevaluable for NE because they had not yet achieved NE or been followed until at least Rel Day 43.

Primary or secondary NE failure was experienced by 10/38 (26.3%) evaluable subjects by Month 24 in TP-103, all of whom were NMSD subjects. Six of these subjects had primary engraftment failure and 4 subjects had secondary engraftment failure. Nine of these subjects went on to receive second allo-HSCTs, and 1 subject underwent a third allo-HSCT after experiencing primary engraftment failure after both first and second allo-HSCTs.

Table 25 Neutrophil engraftment (TP-102/104, TP-103) (Evaluable Patients, Day 120 Update)

	Eli-cel	Allo-HSCT[a]
	TP-102/104 (N = 51)	TP-103 Overall (N = 59)
Neutrophil engraftment by Rel Day 43		
Number of subjects evaluable[b]	49	59
Subjects with NE after first HSCT, n (%)	49 (100.0)	53 (89.8)
Exact 95% CI	92.7, 100.0	79.2, 96.2
Relative Day of neutrophil engraftment		
n	49	53
Median	13.0	17.0
Min, Max	11, 41	12, 36
Primary neutrophil engraftment failure		
n (%)	0	6 (10.2)
Exact 95% CI	0.0, 7.3	3.8, 20.8
Secondary neutrophil engraftment failure by Month 24		
Number of subjects evaluable[c]	27	32
n (%)	0	4 (12.5)
Exact 95% CI	0.0, 12.8	3.5, 29.0
Primary or secondary neutrophil engraftment failure by Month 24		
Number of subjects evaluable[d]	27	38
n (%)	0	10 (26.3)
Exact 95% CI	0.0, 12.8	13.4, 43.1

Source: Interstudy Tables 3.3.1, data cut date 23 October 2020 (Study ALD-102) and 09 October 2020 (Study ALD-104).
Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CI, confidence interval
Note: Parameters are only reported post-first allo-HSCT for Study ALD-103 subjects.
[a] Analysis of NE includes data from subjects after first allo-HSCT only.
[b] Evaluable subjects include those who had NE before Rel Day 43, who discontinued or were lost to follow-up before Rel Day 43 without achieving NE, or who were followed to at least Rel Day 43.
[c] Evaluable subjects include those who achieved NE and either had secondary engraftment failure or had been followed for at least 24 months if no events.
[d] Evaluable subjects include those who were evaluable for primary engraftment failure or secondary engraftment failure.

Thus, as of the data cuts, all subjects infused with Skysona have had successful neutrophil engraftment, and none have required rescue cell therapy.

The applicant also provided additional and updated data presentations that also included all treated patients (not just those considered 'evaluable' according to the SAP) and time to event methodology. These additional presentations were consistent with these data (see Table below).

Forty-nine of 51 Skysona treated subjects achieved NE; 2 subjects from Study ALD-104 had not yet achieved NE or been followed to at least Rel Day 43. No subjects treated with Skysona experienced either primary or secondary neutrophil engraftment failure, compared to 10/59 (16.9%) subjects treated with allo-HSCT, which included 6/59 (10.2%) subjects with primary engraftment failure and 4/59 (6.8%) subjects with secondary engraftment failure.

Table 26 Neutrophil engraftment (TP-102/104, TP-103) (TP Population, Day 120 Update)

	Eli-cel	Allo-HSCT[a]
	TP-102/104 (N = 51)	TP-103 Overall (N = 59)
Neutrophil engraftment by Rel Day 43		
Subjects with NE after first HSCT, n (%)	49 (96.1)	53 (89.8)
Exact 95% CI	86.5, 99.5	79.2, 96.2
Relative Day of neutrophil engraftment		
Median	13.0	17.0
Min, Max	11, 41	12, 36
Primary neutrophil engraftment failure		
n (%)	0	6 (10.2)
Exact 95% CI	0.0, 7.0	3.8, 20.8
Secondary neutrophil engraftment failure		
n (%)	0	4 (6.8)
Exact 95% CI	0.0, 7.0	1.9, 16.5
Primary or secondary neutrophil engraftment failure		
n (%)	0	10 (16.9)
Exact 95% CI	0.0, 7.0	8.4, 29.0

Source: Table 44.3.3.1a, data cut date 23 October 2020 (Study ALD-102) and 09 October 2020 (Study ALD-104).
Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CI, confidence interval
Note: Parameters are only reported post-first allo-HSCT for Study ALD-103 subjects.
Note: Denominator for percentage is the number of all subjects in the cohort unless otherwise noted.
[a] Analysis of NE includes data from subjects after first allo-HSCT only.

It was explored post-hoc whether changes in standard of care in allo-HSCT treated subjects over time had impacted the rates of NE failure in this population. However, 6 of 13 (46.2%) evaluable subjects treated after June 30, 2016 in the TP-103 population experienced primary or secondary engraftment failure by Month 24, ie a higher rate than in the overall TP-103 population (10/38 [26.3%]. These subjects all had no matched sibling donor.

In TP-102/104, updated safety information shows all evaluable subjects (47/47 [100%]) had successful PE, with a median (min, max) PE on Rel Day 32 (14, 108). Four subjects from Study ALD-104 were considered unevaluable for PE because they had not yet achieved PE or been followed for at least 24 months.

In TP-103, 12 of 59 subjects were not considered evaluable for PE by Month 24 because they had primary or secondary engraftment failure or died. The 47 evaluable subjects all had successful PE, with median (min, max) PE occurring on Rel Day 26 (13, 67).

Additional and updated analyses that also include all treated patients and time to event methodology showed consistent results (see Table below).

Table 27 Platelet Engraftment for the Transplant Population (TP-102/104, TP-103) (TP Population, Day 120 Update)

	Eli-cel	Allo-HSCT[a]
	TP-102/104 (N = 51)	TP-103 Overall (N = 59)
Platelet engraftment		
Subjects who achieved platelet engraftment after first HSCT, n (%)	47 (92.2)	47 (79.7)
Exact 95% CI	81.1, 97.8	67.2, 89.0
Relative Day of platelet engraftment		
Median	32.0	26.0
Min, Max	14, 108	13, 67

Source: Table 44.3.3.1a, data cut date 23 October 2020 (Study ALD-102) and 09 October 2020 (Study ALD-104).
Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CI, confidence interval
Note: Parameters are only reported post-first allo-HSCT for Study ALD-103 subjects.
Note: Denominator for percentage is the number of all subjects in the cohort unless otherwise noted.
[a] Analysis of PE includes data from subjects after first allo-HSCT only.

- ***Common AEs***

Table 28 AEs in ≥10% of subjects by SOC, PT, and study period (TP-102/104) (Initial MAA)

System Organ Class Preferred Term	M to < C (N = 45) n (%), Events	C to < NE (N = 45) n (%), Events	NE to M24 (N = 45) n (%), Events	> M12 to M24 (N = 31) n (%), Events	D1 to M12 (N = 45) n, (%) Events	D1 to LFU (N = 45) n (%), Events
Subjects with at least 1 AE	**39 (86.7), 111**	**45 (100.0), 816**	**41 (91.1), 241**	**8 (25.8), 20**	**45 (100.0), 753**	**45 (100.0), 788**
Blood and lymphatic system disorders	**6 (13.3), 9**	**45 (100.0), 280**	**16 (35.6), 52**	**0**	**45 (100.0), 297**	**45 (100.0), 297**
Thrombocytopenia	1 (2.2), 1	42 (93.3), 81	6 (13.3), 11	0	44 (97.8), 91	44 (97.8), 91
Anaemia	4 (8.9), 5	40 (88.9), 73	6 (13.3), 9	0	38 (84.4), 65	38 (84.4), 65
Neutropenia	0	37 (82.2), 54	10 (22.2), 17	0	38 (84.4), 66	38 (84.4), 66
Febrile neutropenia	0	35 (77.8), 41	0	0	35 (77.8), 40	35 (77.8), 40
Leukopenia	2 (4.4), 3	11 (24.4), 24	4 (8.9), 13	0	11 (24.4), 30	11 (24.4), 30
Lymphopenia	0	6 (13.3), 6	0	0	2 (4.4), 2	2 (4.4), 2
Gastrointestinal disorders	**11 (24.4), 15**	**45 (100.0), 218**	**18 (40.0), 23**	**1 (3.2), 1**	**45 (100.0), 127**	**45 (100.0), 130**
Stomatitis	0	39 (86.7), 48	0	0	37 (82.2), 45	37 (82.2), 45
Vomiting	4 (8.9), 4	35 (77.8), 49	6 (13.3), 7	0	14 (31.1), 18	15 (33.3), 19
Abdominal pain	1 (2.2), 1	20 (44.4), 23	2 (4.4), 2	0	13 (28.9), 16	14 (31.1), 17
Diarrhoea	0	16 (35.6), 17	2 (4.4), 2	1 (3.2), 1	12 (26.7), 13	13 (28.9), 14
Nausea	7 (15.6), 7	41 (91.1), 50	4 (8.9), 5	0	11 (24.4), 14	11 (24.4), 14
Constipation	1 (2.2), 1	15 (33.3), 15	5 (11.1), 5	0	7 (15.6), 7	7 (15.6), 7
Skin and subcutaneous tissue disorders	**6 (13.3), 8**	**32 (71.1), 46**	**15 (33.3), 21**	**0**	**37 (82.2), 59**	**37 (82.2), 59**
Alopecia	0	26 (57.8), 26	8 (17.8), 8	0	34 (75.6), 34	34 (75.6), 34
Pruritus	3 (6.7), 3	5 (11.1), 5	1 (2.2), 1	0	5 (11.1), 5	5 (11.1), 5
Skin hyperpigmentation	0	1 (2.2), 1	5 (11.1), 5	0	5 (11.1), 5	5 (11.1), 5
Rash	1 (2.2), 1	6 (13.3), 6	1 (2.2), 1	0	4 (8.9), 4	4 (8.9), 4
Metabolism and nutrition disorders	**9 (20.0), 11**	**36 (80.0), 94**	**7 (15.6), 9**	**1 (3.2), 1**	**26 (57.8), 53**	**27 (60.0), 54**
Decreased appetite	0	32 (71.1), 40	2 (4.4), 2	0	16 (35.6), 18	16 (35.6), 18
Hypokalaemia	5 (11.1), 5	21 (46.7), 25	2 (4.4), 2	0	12 (26.7), 14	12 (26.7), 14
Hypophosphataemia	0	7 (15.6), 7	2 (4.4), 2	0	8 (17.8), 9	8 (17.8), 9
Hypomagnesaemia	5 (11.1), 5	5 (11.1), 5	1 (2.2), 1	0	2 (4.4), 2	2 (4.4), 2

System Organ Class Preferred Term	M to < C (N = 45) n (%), Events	C to < NE (N = 45) n (%), Events	NE to M24 (N = 45) n (%), Events	> M12 to M24 (N = 31) n (%), Events	D1 to M12 (N = 45) n, (%) Events	D1 to LFU (N = 45) n (%), Events
Respiratory, thoracic and mediastinal disorders	**1 (2.2), 1**	**15 (33.3), 19**	**7 (15.6), 7**	**1 (3.2), 1**	**21 (46.7), 23**	**21 (46.7), 24**
Epistaxis	0	8 (17.8), 9	1 (2.2), 1	0	9 (20.0), 9	9 (20.0), 9
Cough	0	4 (8.9), 4	2 (4.4), 2	0	6 (13.3), 6	6 (13.3), 6
General disorders and administration site conditions	**22 (48.9), 29**	**16 (35.6), 22**	**13 (28.9), 18**	**1 (3.2), 1**	**19 (42.2), 28**	**20 (44.4), 32**
Pyrexia	3 (6.7), 3	9 (20.0), 9	11 (24.4), 16	1 (3.2), 1	14 (31.1), 19	15 (33.3), 22
Catheter site pain	14 (31.1), 20	2 (4.4), 2	0	0	0	0
Nervous system disorders	**2 (4.4), 2**	**14 (31.1), 16**	**13 (28.9), 26**	**4 (12.9), 6**	**15 (33.3), 28**	**19 (42.2), 38**
Headache	1 (2.2), 1	10 (22.2), 11	3 (6.7), 3	0	8 (17.8), 8	8 (17.8), 8
Investigations	**5 (11.1), 5**	**14 (31.1), 41**	**5 (11.1), 8**	**0**	**10 (22.2), 24**	**10 (22.2), 24**
Alanine aminotransferase increased	0	8 (17.8), 9	2 (4.4), 2	0	6 (13.3), 6	6 (13.3), 6
Aspartate aminotransferase increased	0	7 (15.6), 9	1 (2.2), 1	0	4 (8.9), 5	4 (8.9), 5
Vascular disorders	**1 (2.2), 1**	**10 (22.2), 13**	**2 (4.4), 2**	**0**	**8 (17.8), 10**	**8 (17.8), 10**
Hypertension	1 (2.2), 1	6 (13.3), 7	2 (4.4), 2	0	4 (8.9), 5	4 (8.9), 5

Source: Interstudy Table 3.4.8

Abbrev.: AE, adverse event; C, conditioning; LFU, Last Follow-up; M, mobilization; NE, neutrophil engraftment; PT, preferred term; SOC, system organ class

Note: PTs (and their associated SOC) are included for AEs that were observed in ≥10% of subjects (≥5 subjects) in any shown study period and are sorted based on decreasing frequency by SOC and then PT per the **D1 to LFU** study period. For such PTs the frequency of AEs are shown even if they occurred in <10% of subjects in some study periods. The SOC values presented show the incidence of all subjects/events that occurred under that SOC (not only those events meeting the ≥10% threshold).

Note: Subjects at risk for each period (N in column header) is defined to be the subjects who entered the study period. If event started in one reporting period and continued into the next reporting period, it was counted only in the first period. If event started and stopped in one reporting period and then recurred in the next reporting period, it was counted in both periods. Subjects were counted once for each SOC and PT even if they had multiple instances of the event in one period. For AEs with worsening severity in which the AE started in the first period and worsened in the next period, the subject was counted in both periods. All events reported in the database are counted in the number of events.

Note: Hematologic abnormalities reported as AEs that were coded to PTs in the Investigations SOC (e.g., platelet count decreased) have been pooled with appropriate terms in the Blood and Lymphatic System SOC (e.g., thrombocytopenia) for tabulation.

Adverse Events Attributed to Mobilisation/Apheresis

Twenty-three of the 45 subjects (51.1%) experienced 36 AEs attributed to mobilisation/apheresis by the Investigator and all of these were reported in the Mobilisation to Conditioning period. The most frequently reported SOCs and PTs included:

- Metabolism and nutrition disorders (8/45 [17.8%]): The most frequently reported PTs included Hypomagnesaemia and Hypokalaemia, each in 5 subjects.
- Gastrointestinal disorders (7/45 [15.6%]): The most frequently reported PTs included Nausea in 4 subjects and Vomiting in 3 subjects.
- Blood and lymphatic system disorders (5/45 [11.1%]): The most frequently reported PT was Anaemia in 4 subjects.
- Musculoskeletal and connective tissue disorders (5/45 [11.1%]): The most frequently reported PT was Bone pain in 4 subjects.

All AEs attributed to mobilisation/apheresis were nonserious and all were Grade 1 or 2 in severity except for one Grade 3 AE of Hypokalaemia.

Adverse Events Attributed to Conditioning

The vast majority of the reported AEs in TP-102/104 were attributed to conditioning by the investigator. For example, 45 subjects reported 816 AEs in the C to <NE period, of which 684 events were deemed to be related to the conditioning.

AEs were most frequently reported in the following SOCs (percentages provided reflect C to < NE incidence): Gastrointestinal disorders (45/45 [100%]), Blood and lymphatic system disorders (44/45 [97.8%]), Metabolism and nutrition disorders (36/45 [80.0%]), and Skin and subcutaneous tissue disorders (28/45 [62.2%]). Subjects most frequently experienced AEs of Nausea (41/45 [91.1%]), Thrombocytopenia (41/45 [91.1%]), Anaemia (39/45 [86.7%]), Stomatitis (39/45 [86.7%]), and Neutropenia (37/45 [82.2%]). The distribution of the PTs was similar between the 2 studies considering the small patient numbers.

The applicant discussed whether any relevant differences in safety profile were observed between the 2 conditioning regimens used between the study ALD-102 (busulfan and cyclophosphamide) and ALD-104 (busulfan and fludarabine). Data to date reflect, in general, a comparable profile between the conditioning regimens used given the small population and the characteristics of the used agents. However, 4 of the 19 subjects in the ongoing Study ALD-104 were not yet available for PE as of the data cut and there were at least 3 subjects with relatively long times to PE: these subjects in Study ALD-104 achieved PE after Rel Day 60 (Rel Day 104, 108, and 106 respectively). In addition, two of these 3 subjects reported SAEs of pancytopenia (still ongoing). Given the limited safety data available, a full B/R evaluation on this conditioning regimen is not yet possible.

The majority of events were non-serious. In the initial MAA, 17 subjects experienced 26 SAEs attributed to conditioning, however (all of which resolved) that included 11 events of Febrile neutropenia, 8 events of Pyrexia, and 1 event each of Stomatitis, Otitis media, Decreased appetite, Device related infection, Streptococcus bacteraemia, Oral mucositis, and Constipation.

There is close proximity in time, however, of Skysona administration in relation to the conditioning regimen (once the cyclophosphamide regimen was completed, this was followed by 1 rest day before Skysona infusion). In total, 532 of the 816 AEs that were overall reported in the period C to <NE (regardless of causality) were reported during or after D1. Of the 684 events attributed to conditioning by the investigator, 447 were reported during or after D1.

A side-by-side presentation of AEs (by SOC and PT) that emerged in the period 'C to D1' vs. 'D1 to <NE', respectively and a discussion of any patterns of interest was provided but did not reveal any new safety concerns.

Adverse Events Treatment-Emergent to Skysona

In the initial MAA for TP-102/104, all subjects (45/45) experienced at least 1 TEAE (occurring during or after administration of Skysona). A total of 5/45 (11.1%) subjects had TEAEs that were considered by the Investigator to be drug product related TEAEs.

Two events were non-serious Grade 1 AEs of Vomiting that started and resolved on Rel Day 1, likely related to DMSO used as a cryopreservative in Skysona drug product. Three events were SUSARs: one case of Grade 3 SAE of viral cystitis in ALD-102 due to BK virus from Rel Day 42 to 48, which resolved with supportive treatment; and 2 SAEs of pancytopenia due to delayed haematopoietic reconstitution in ALD-104. Enrolment in this study was halted in October 2019 but resumed in February 2020.

Both SAEs were considered to be possibly related to Skysona and are still ongoing events. The role of alternative possible causative factors (parvovirus, immune-related causes) remains unclear. Both subjects are clinically stable, however, with below-normal platelet levels.

Post-engraftment cytopenias that required growth factor treatment were reported. Thirty of 45 subjects in TP-102/104 received G-CSF in the period after NE (D120 Listing 46.2.8.19.3). In 22 of these subjects, G-CSF was administered continuously from prior to NE to shortly after NE. In general, post engraftment use of G-CSF was brief in duration and often associated with a clinical finding (e.g., febrile neutropenia) with the exception of the 2 subjects who experienced delayed haematopoietic reconstitution. In these subjects, G-CSF was administered more than 100 days after the subjects achieved NE. These two subjects received eltrombopag due to decreased platelets.

All 45 patients were treated with antibiotic during the conditioning therapy (thus C to <NE). The majority (39, 86.7%) were treated with antibiotic due to an adverse event however, in the majority (n=35) of these patients, the adverse event was not directly related to an infection but rather a symptomatic treatment in cases where infections could not be excluded (diagnoses of e.g. 'Pyrexia', or 'Febrile neutropenia'.

Section 4.4 of the SmPC has been updated to include warning for prolonged cytopenia and this has also been included in the safety specification as an important identified risk.

- ***Comparison of ≥Grade 3 TEAEs: Skysona and Allo-HSCT***

AE collection between TP-102/104 and TP-103 was considered only comparable for≥ Grade 3 TEAEs through Month 12 (excluding haematological toxicities related to conditioning medication), as well as ≥ Grade 3 TEAEs related to HSCT and TESAEs through Month 48.

- In the Vascular disorders SOC, no subjects in TP-102/104 experienced an event, while 28/59 subjects (47.5%) in TP-103 experienced 36 ≥ Grade 3 TEAEs. Twenty nine of these 36 events were Hypertension which was experienced by 28/59 (47.5%) subjects. Of these 28 subjects, 4 were in the MSD subgroup and the remaining 24 subjects were in the NMSD subgroup. Hypertension is most likely a side effect of the immunosuppressant cyclosporine which is commonly used for post-allo-HSCT immunosuppression.
- In the Infections and infestations SOC, 4 of 45 subjects (8.9%) in TP-102/104 experienced 6 ≥ Grade 3 TEAEs, whereas 34 of 59 subjects (57.6%) in TP-103 experienced 78 events. More subjects experienced ≥ Grade 3 TEAEs of infection attributed to conditioning in TP-103 (16/59 [27.1%]) as compared to TP-102/104 (2/45 [4.4%]) which may be related to differences in the respective conditioning regimens used or difference in baseline characteristics.

- In the Immune system SOC, no subjects in TP-102/104 experienced an event while 7/59 (11.9%) subjects in TP-103 experienced 8 ≥ Grade 3 TEAEs, including Engraftment syndrome and Transplant rejection. The majority of these events were reported in the NMSD subgroup.

- ***Adverse Events of Special Interest***

GvH disease

No evaluable subjects (0/31) in TP-102 experienced either acute (≥ Grade II) or chronic GVHD by Month 24, compared to 26/50 (52%) subjects in TP-103 (see table and figure below).

In the TPES-103 population, 10/27 (41.7%) developed either acute GVHD (≥Grade II) or chronic GVHD by Month 24 but for the subgroup of TPES-103 MSD patients (N=10), there were three (30.0%).

Table 29 Proportion of subjects with either acute GVHD (≥ Grade II) or chronic GVHD by Month 24 – main analysis (TP-102, TP-103) (Initial MAA, evaluable patients)

	Eli-cel	Allo-HSCT[a]		
	TP-102 (N = 32)	TP-103 Overall (N = 59)	TP-103 MSD (N = 11)	TP-103 NMSD (N = 48)
Subjects with acute GVHD (≥ Grade II) by Month 24				
Evaluable subjects	31	49	10	39
n (%)	0	15 (30.6)	1 (10.0)	14 (35.9)
Exact 95% CI	0.0, 11.2	18.3, 45.4	0.3, 44.5	21.2, 52.8
p-value[b]		0.0003	0.2439	0.0001
Subjects with chronic GVHD by Month 24				
Evaluable subjects	31	39	9	30
n (%)	0	14 (35.9)	2 (22.2)	12 (40.0)
Exact 95% CI	0.0, 11.2	21.2, 52.8	2.8, 60.0	22.7, 59.4
p-value[b]		0.0001	0.0462	<0.0001
Subjects with either acute GVHD (≥ Grade II) or chronic GVHD by Month 24				
Evaluable subjects	31	50	10	40
n (%)	0	26 (52.0)	3 (30.0)	23 (57.5)
Exact 95% CI	0.0, 11.2	37.4, 66.3	6.7, 65.2	40.9, 73.0
p-value[b]		<0.0001	0.0113	<0.0001

Source: Interstudy Table 3.1.1.1, Table 3.1.1.1, and Table 3.1.1.1.2

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CI, confidence interval; GVHD, graft-versus-host disease; MSD, matched sibling donor; NMSD, not a matched sibling donor

Note: Evaluable subjects are defined as those who had the respective event by Month 24 (Rel Day 730) in any allo-HSCT Period or had been followed for at least 12 months (DLC Rel Day >= 365 days) in the latest allo-HSCT Period if no events.

[a] Analysis of GVHD includes data from subjects who underwent more than one allo-HSCT. Undergoing rescue cell administration /subsequent allo-HSCT(s) was not considered as events in this analysis.

[b] P-values is based on Fisher's exact test.

At the Day 120 data update, consistent with the data presented in the initial MAA, no Skysona treated subject (0/32 [0%]) in Study ALD-102 experienced acute (≥ Grade II) or chronic GVHD by Month 24 compared to 26/50 (52%) evaluable subjects treated with allo-HSCT in Study ALD-103.

For the TPES-103 population (N=27), 10/24 (41.7%) evaluable subjects experienced acute (≥ Grade II) or chronic GVHD by Month 24.

Since no subjects experienced acute or chronic GVHD as of the updated data cuts, the median time to first acute or chronic GVHD continued not to be estimable and the data from TP-103 were unchanged from the original MAA.

An updated analysis of GVHD, using data from Q4 2020 for the entirety of the TP rather than subjects as defined in the SAP showed that no subjects treated with Skysona (TP-102/104) experienced either acute (≥ Grade II) or chronic GVHD. In contrast, among subjects who received allo-HSCT, 26/59 (44.1%) subjects in TP-103 and 10/27 (37.0%) subjects in TPES-103 experienced either acute (≥ Grade II) or chronic GVHD.

Table 30 Proportion of Subjects with either acute GVHD (≥ Grade II) or chronic GVHD using – TP-102/104, TPES-103, TP-103 (TP Population, Day 120 Update)

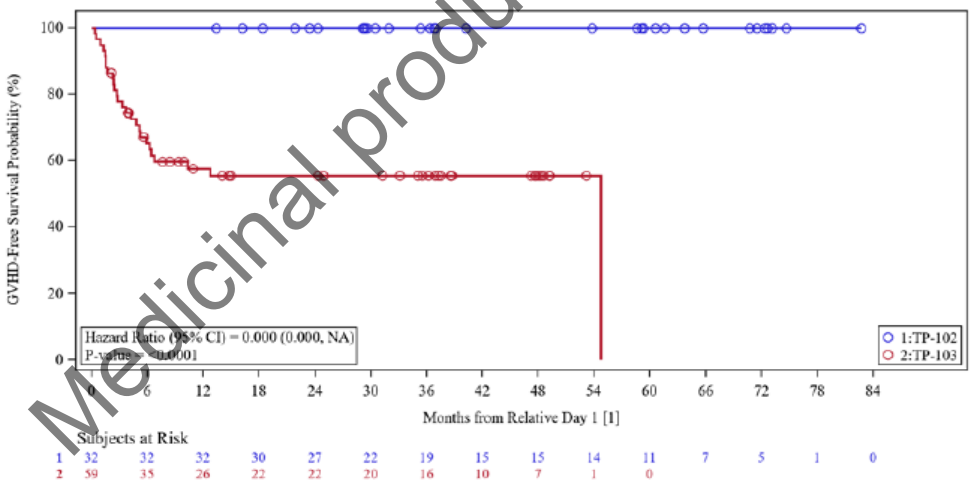
	Eli-cel		Allo-HSCT[a]	
	TP-102 (N = 32)	TP-102/104 (N = 51)	TPES-103 (N = 27)	TP-103 Overall (N = 59)
Subjects with acute GVHD (≥ Grade II)				
n (%)	0	0	5 (18.5)	15 (25.4)
Exact 95% CI	0.0, 10.9	0.0, 7.0	6.3, 38.1	15.0, 38.4
Subjects with chronic GVHD				
n (%)	0	0	7 (25.9)	14 (23.7)
Exact 95% CI	0.0, 10.9	0.0, 7.0	11.1, 46.3	13.6, 36.6
Subjects with either acute GVHD (≥ Grade II) or chronic GVHD				
n (%)	0	0	10 (37.0)	26 (44.1)
Exact 95% CI	0.0, 10.9	0.0, 7.0	19.4, 57.6	31.2, 57.6

Source: Table 44.3.1.1.1a and Table 44.3.1.1.1c, data cut date 23 October 2020 (Study ALD-102) and 09 October 2020 (Study ALD-104)

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CI, confidence interval; GVHD, graft-versus-host disease; TP, transplant population; TPES, strictly ALD-102 eligible population

[a] Analysis of GVHD includes data from subjects who underwent more than one allo-HSCT. Undergoing rescue cell administration /subsequent allo-HSCT(s) was not considered as events in this analysis.

Table 31 Time to First Acute GVHD (≥ Grade II) or Chronic GVHD, Kaplan-Meier Analysis (TP-102, TP-103) (Day 120 Update)



Source: Interstudy Figure 3.1.2.1, data cut date 23 October 2020 (Study ALD-102)

Abbrev.: CI, confidence interval; GVHD, graft-versus-host disease; TP, Transplant Population.

Note: Estimates of acute or chronic GVHD-free survival time are obtained using the Kaplan-Meier method, where events include acute (≥ Grade II) or chronic GVHD.

Note; Symbol 'o' represents censoring. Subjects who did not experience any event are censored at their date of last contact.

Note: Time to event analysis of GVHD includes data from subjects who underwent more than one allo-HSCT. Undergoing rescue cell administration /subsequent allo-HSCT (s) was not considered as events in this analysis.

Note: The hazard ratio (95% CI) is based on Cox regression model, and p-value is based on log-rank test.

In an additional competing risk sensitivity analysis with engraftment failure (primary and secondary), transplant related mortality, and death as competing risks. No GVHD events were observed by Month 24 in TP-102 resulting in an estimated cumulative incidence function (CIF) of 0% at Month 24 as compared to the estimated CIF of 37.8% (95% CI: 25.0, 50.5) in TP-103. The CIF estimate for TP-103 MSD (N=11) was 29.3% (95% CI: 6.0, 58.4) and for TP-103 NMSD (N=48): 39.4 (25.1, 53.4).

Treatment-emergent infections

In TP-102/104, 20/45 (44.4%) subjects experienced TEAEs in the Infections and infestations SOC, predominantly during NE to M12 (15/45 [33.3%]).

The majority of infections in TP-102/104 were nonserious, not opportunistic, and either self-limited or resolved with standard therapy. None of the infections except for a case of viral cystitis was considered related to Skysona by the investigator.

In Study TP-103, there were 4 fatal infections experienced by 3 subjects.

Other events of interest

At the time of the interim data cut, there were no AEs or events of interest attributable to the use of an LVV for the ex vivo transduction of autologous cells, i.e. no confirmed detection of replication of competent lentivirus (RCL), or malignancy.

However, one subject in the ongoing Study LTF-304 (who received Skysona in Study ALD-102) has shown a gradual, persistent expansion of a single clone. Integration site analyses (ISA) has shown a persistent high relative IS-frequency of 3 IS (with one of the IS in the *MECOM* gene) in a single clone over time, with a relative clonal contribution of ~14% in PBLs and ~41% in myeloid cells at Month 60 and 49% to 59% at Month 65 (see table below). As of the Year 6 evaluation, samples collected during Year 6 Visit qPCR analyses showed that IS-specific VCN estimated a clonal contribution of ~30% in peripheral blood leukocytes and ~77% in myeloid lineage (CD15+ cells).

Since MAA submission, one additional subject from Study ALD-104 has developed a predominant clone with 4 IS, including an IS in *MECOM*.

Transcription analysis of these subjects reveals dysregulation of the *MECOM* gene, resulting in increased levels of a subset of *MECOM* transcripts.

Both subjects continue to be followed but remain clinically stable with no evidence of malignancy (see also Section Laboratory findings for further details on ISA analyses).

Insertional oncogenesis is included in the safety specification as important potential risk.

Table 32 Subjects Treated with Skysona who Have a Predominant Clone (Day 120 Update)

Subject	Gene name (s) in which insertions were identified in the predominant clone	Visits and cell populations in which a predominant clone was identified	Cell type and clonal contribution at most recent ISA[a]
	ACER3, RFX3, MECOM	**Month 65** [b]: CD15+ cells	**Month 65 CD15+ cells**: 49% to 59%
	ACTR3, MECOM, RAP2C-AS1, ST3GAL6-AS1	**Month 6** [c]: PBLs **Month 12** [d]: PBLs and CD15+ cells	**Month 12 PBLs**: 64% to 81% **Month 12 CD15+ cells**: 80% to 90%

[a] Clonal contribution for each IS was determined separately (result calculated as copies per diploid genome [c/dg], then multiplied by 100 to express contribution of the clone carrying this insertion as a percentage of the population of cells assayed), then the range is provided to give an estimate of the percentage clonal contribution (assuming all IS were present in the same clone).
[b] This was an Unscheduled Visit on 29 July 2020 at approximately 65.7 months after drug product infusion, and is denoted as Month 65 Visit for this document.
[c] Scheduled Month 6 Visit on 06 January 2020 (approximately 6 months after drug product infusion)
[d] Scheduled Month 12 Visit on 10 August 2020 (approximately 13 months after drug product infusion)

- ***Long-term safety - adverse events in Study ALD-304***

In the initial MAA, results were presented for the 21 enrolled subjects from Study ALD-102 as of the data cut date (31 January 2020). Subjects from Study ALD-104 have not yet enrolled. Subject are to be followed through to Year 15 post-drug product infusion (the parent study includes approximately 2 years follow-up after drug product infusion) and the current median follow-up time is 56 months.

As of the cut-off date, no subjects experienced GVHD or underwent a second stem cell transplantation.

No deaths or AEs related to Skysona have been reported during this study.

Seven SAEs in 4 subjects were reported: 3 SAEs of Seizure, and 1 SAE each of Fatigue, Depression, Suicidal ideation, and Pyrexia. None of the events were considered related to Skysona.

The Day 120 Safety Update revealed 2 additional SAEs in this study: both SAEs of Seizure and not related to Skysona. As stated above, ISA revealed 1 subject in LTF-304 with a predominant clone.

Serious adverse events and deaths

One death (1/45, 2.2%) was reported in TP-102/104. Subject experienced rapid CALD disease progression starting 2 weeks after treatment with Skysona (Loes score increase from 6.5 at Baseline to 13.5 at Day 14) with SAEs of Neurological decompensation eventually followed by Cardio-respiratory arrest on Rel Day 666.

One additional subject died due to complications of allogeneic transplantation after receiving allo-HSCT off study.

Neither of the deaths were considered related to Skysona.

Up to the interim cut-off date, 15/59 (25.4%) subjects died in TP-103 (12 after first allo-HSCT and 3 after second allo-HSCT), 9 of whom had transplant-related mortality (TRM). The 6 non-TRM deaths were attributed to progressive disease/CALD in 3 subjects, and in 3 subjects the cause of death or attribution was unknown.

Two of the 15 deaths occurred in MSD subjects. One case was attributed to GVHD. The other subject died from Septic shock attributed to unknown cause.

No deaths were reported in LFT-304 up to the cut-off date for the interim analysis.

In TP-102/104, 27/45 (60%) subjects experienced 63 TESAEs from D1 through Last Follow-up. the overall SAE profile was largely consistent with the effects of conditioning. The most frequently experienced TESAEs were related to Blood and lymphatic system disorders (12/45 [26.7%]), General disorders and administration site conditions (10/45 [22.2%]), Infections and infestations (7/45 [15.6%]), and Gastrointestinal disorders (5/45 [11.1%]).

Three subjects had SAEs that were considered possibly related to Skysona.

Two of the SAEs that were assessed by the Investigator to be possibly related to Skysona were the events of pancytopenia previously discussed (from study ALD-104).

In study ALD-102, one SAE, an event of Cystitis viral (Grade 3), was assessed by the Investigator as possibly related to Skysona. This subject (with a medical history of adrenal insufficiency), was diagnosed with BK virus haemorrhagic cystitis on Rel Day 44 and fully recovered following symptomatic treatment. Overall, a causal relationship to Skysona seems unlikely.

All TESAEs resolved with the exception of the SAEs ongoing at the time of death in one subject, the SAEs of pancytopenia in two subjects. Updated narratives reported that both events of pancytopenia are not resolved. One of the subjects was treated with immunosuppressants with no response. Both subjects were clinically stable with no need for platelet transfusions or eltrombopag but still had below-normal platelet levels.

The reported cases of pancytopenia are reflected in the SmPC.

In addition, there is an ongoing SAE of Grade 3 myelitis transverse in one subject. The event was considered not or unlikely related to Skysona by the investigator. The aetiology remains unclear but there was no evidence of an immunogenic reaction.

The proportion of subjects with serious infections was more than 2-fold higher in TP-103 than in TP-102/104 (22/59 [37.3%] vs 7/45 [15.6%], respectively), and the number of serious infections was more than 5-fold higher (47 events vs 8 events, respectively). Many serious infections in TP-103 were opportunistic (14 SAEs), reflective of the subjects' chronically immunocompromised state, while 1 serious opportunistic infection was reported in TP-102/104 (Cystitis viral). The majority of TESAE infections (38/47 events) in TP-103 were in 16 subjects in the NMSD subgroup.

There were no TESAEs reported in the Vascular disorders SOC for TP-102/104; however, 7/59 (11.9%) subjects in TP-103 experienced 8 TESAEs in this SOC, with 6 of these 7 subjects in the NMSD subgroup. Two subjects had serious events of hypertension, a known consequence of immunosuppressants, and 4 subjects had 4 events that were thrombotic (e.g., cerebral infarction, deep vein thrombosis, thrombosis, and veno-occlusive disease), which may be linked the endothelial effects of immunosuppression.

Two subjects in TP-103 experienced 3 TESAEs attributed to autoimmunity by the Investigator. One NMSD subject experienced a TESAE of Autoimmune haemolytic anaemia and an MSD subject experienced 2 TESAEs attributed to autoimmunity by the Investigator, haemolytic anaemia and encephalopathy.

Table 33 Serious Adverse Events by SOC, PT, and Study Period (TP-102/104, TP-103)

System Organ Class	TP-102/104 (N = 45) n (%), Events	TP-103		
		Overall (N = 59) n (%), Events	MSD (N = 11) n (%), Events	NMSD (N = 48) n (%), Events
Subjects with ≥1 TESAE to M48	**27 (60.0), 60**	**43 (72.9), 158**	**8 (72.7), 21**	**35 (72.9), 137**
Infections and infestations	7 (15.6), 8	22 (37.3), 47	6 (54.5), 9	16 (33.3), 38
Blood and lymphatic system disorders	12 (26.7), 13	12 (20.3), 23	2 (18.2), 2	10 (20.8), 21
Nervous system disorders	3 (6.7), 9	11 (18.6), 25	2 (18.2), 2	9 (18.8), 23
General disorders and administration site conditions	8 (17.8), 11	8 (13.6), 9	2 (18.2), 2	6 (12.5), 7
Vascular disorders	0	7 (11.9), 8	1 (9.1), 1	6 (12.5), 7
Gastrointestinal disorders	5 (11.1), 5	5 (8.5), 7	3 (27.3), 4	2 (4.2), 3
Immune system disorders	0	4 (6.8), 5	0	4 (8.3), 5
Metabolism and nutrition disorders	1 (2.2), 1	4 (6.8), 7	0	4 (8.3), 7
Respiratory, thoracic and mediastinal disorders	1 (2.2), 1	4 (6.8), 7	0	4 (6.8), 7
Ear and labyrinth disorders	0	3 (5.1), 3	1 (9.1), 1	2 (4.2), 2
Injury, poisoning and procedural complications	3 (6.7), 3	3 (5.1), 3	0	3 (6.3), 3
Renal and urinary disorders	2 (4.4), 2	3 (5.1), 5	0	3 (6.3), 5
Psychiatric disorders	2 (4.4), 3	1 (1.7), 1	0	1 (2.1), 1
Investigations	1 (2.2), 1	2 (3.4), 2	0	2 (4.2), 2
Endocrine disorders	0	2 (3.4), 3	0	2 (4.2), 3
Cardiac disorders	1 (2.2), 1	1 (1.7), 3	0	1 (2.1), 3
Hepatobiliary disorders	1 (2.2), 1	0	0	0
Musculoskeletal and connective tissue disorders	1 (2.2), 1	0	0	0

Shortened by Assessor. Source: Interstudy Table 3.4.2.1, Table 3.4.2.1.1 and 3.4.2.1.2

Note: PTs (and their associated SOC) are included for all SAEs that were observed in the D1 to M48 study period and are sorted based on decreasing frequency by SOC and then PT (and then alphabetically) based on TP-103 overall. Subjects at risk for each period (N in column header) is defined to be the subjects who entered the study period. If event started in one reporting period and continued into the next reporting period, it was counted only in the first period. If event started and stopped in one reporting period and then recurred in the next reporting period, it was counted in both periods. Subjects were counted once for each SOC and PT even if they had multiple instances of the event in one period. For SAEs with worsening severity in which the AE started in the first period and worsened in the next period, the subject was counted in both periods. All events reported in the database are counted in the number of events.

In the ongoing study LTF-304, 7 SAEs in 4 subjects were reported in the initial MAA: 3 SAEs of seizure, and 1 each of fatigue, depression, suicidal ideation, and pyrexia. None of the SAEs was considered related to Skysona.

Five additional subjects reported new TESAEs have been reported since the initial MAA data cuts, 3 subjects from the ongoing Study ALD-104 and 2 subjects in the ongoing Study LTF-304 (originally enrolled in ALD-102). The cases in Study ALD-104 referred to Pseudomonal bacteraemia (attributed to conditioning), Pyrexia (attributed to catheter removal) and febrile neutropenia (attributed to conditioning). All events resolved. In Study LTF-304, there were 2 SAEs of seizure, not considered related to Skysona.

In addition, a late-breaking data cut from the safety database on 05 February 2021 identified 5 new SAEs. These were 2 cases of seizure, one case of pyrexia, one case of viral upper respiratory tract infection, and one case of Pseudomonal bacteraemia and Stenotrophomonas infection. None of these events were considered related to Skysona by the Investigator but consistent with the effects of conditioning and/or underlying disease.

Laboratory findings

Severe depletion of neutrophils and platelets was observed during and after conditioning as expected.

In TP-102/104, 10 subjects had non-serious AEs of neutropenia reported within days of NE and 6 subjects had non-serious AEs of thrombocytopenia reported within days of PE. Post-engraftment cytopenias were generally transient and resolved except for the 2 subjects in Study ALD-104 who had ongoing SUSARs of pancytopenia at the time of the interim data cut. One of these subjects remained reportedly stable with no need for platelet transfusions or eltrombopag but the event of pancytopenia was not considered fully resolved at the time of submission. On Rel Day 573, laboratory results showed WBC 3.1 $\times 10^9$ cells/L, ANC 2.1 $\times 10^9$ cells/L, haemoglobin 11.6 g/dL, platelets 27 $\times 10^9$ cells/L. For the other subject, the event is also still ongoing. On Rel Day 510, laboratory results showed WBC 3.6 $\times 10^9$ cells/L, ANC 0.7 $\times 10^9$ cells/L, haemoglobin 13.7 g/dL, platelets 127 $\times 10^9$ cells/L with no need for platelet transfusions or eltrombopag.

In both cases, the grade 3 Pancytopenia was considered at least possibly related to Skysona although other causal factors (the role of parvovirus or potential immune-related causes) could not be excluded.

In TP-102/104, hypokalaemia during the C to NE period was the most common electrolyte disturbance, with PCS (threshold of ≤3 mmol/L) low values noted in 24/45 (53.3%). No subjects evaluable after Month 12 had potassium values meeting low PCS criteria. AE reporting was generally consistent with this observation.

A total of 25 (55.6%) of all patients experienced at least one episode of Grade ≥3 hypokalaemia. The majority of the patients experienced hypokalaemia during the conditioning period (C to NE) and most often hypokalaemia could be attributed to either vomiting or diarrhoea which could likely be an adverse reaction to the conditioning treatment. Overall, hypokalaemia is commonly reported during both the mobilisation/apheresis and the conditioning treatment and this has been reflected in the SmPC.

Detection of Replication Competent Lentivirus

All patients were screened and confirmed to be negative for lenti- and/or retroviral infections as a condition for receiving treatment with Skysona. As of the database lock, there have been no confirmed positive results for LVV-derived RCL in any subject treated with Skysona. In Studies ALD-102 and ALD-104, RCL samples are tested through Month 12 as outlined in the protocol. If all results are negative, subsequent samples in these studies, as well as in the long-term follow up study LTF-304, are collected and archived. The RCL analysis was (or is) not performed routinely during LTF-304 but this has been sufficiently justified.

Integration Site Analysis

Clinical study subjects are being monitored by ISA at 3 months and 6 months after treatment, followed by every 6 months up to 5 years post-drug product infusion, and then at Years 7, 10, and 15.

The initial MAA reported on the IS monitoring performed in the 32 subjects who were enrolled in the open-label clinical Phase 2/3 Study ALD-102 or its long-term follow-up Study LTF-304. In total, >334,400 unique IS were identified.

Among these, IS in the *SMG6* and *MECOM* genes were the most frequently detected in over 190 different samples from all 32 subjects analysed.

In updated results, multiple subjects had at least one IS within *SMG6* (25/27 in Study LTF-304) or within *MECOM* (14/27 in Study LTF-304) in their top 10 genes. Multiple *SMG6* IS was detected in the Top 10 most frequent IS in multiple subjects at multiple time points by screening ISA of peripheral blood, although none reached a relative IS frequency of >30% in more than two consecutive analysis time points. To date, none of these IS have been associated with a predominant clone. Insertion of an MNDU3 promoter-GFP transgene did not result in overexpression of the *SMG6* gene in cultured CD34+ cells.

One subject in Study LTF-304 (who received Skysona in Study ALD-102) has shown a gradual, persistent expansion of a single clone over time. The predominant clone had 3 integration sites (IS) in the genes A*CER3, RFX3,* and *MECOM*.

As of the Year 6 evaluation, samples collected during Year 6 Visit qPCR analyses showed that IS-specific VCN estimated a clonal contribution of ~30% in peripheral blood leukocytes and ~77% in myeloid lineage (CD15+ cells).

Clinically, this subject has normal haemoglobin, platelets, and white blood cell count and a neutrophil count at the lower end of the normal range.

In addition, one subject who was treated with Skysona in Study ALD-104 has been identified with a predominant clone with 4 IS, including an IS in *MECOM* (*ACTR3*, *RAP2C-AS1, ST3GAL6-AS1, and MECOM*). This subject first met the criteria at approximately 6 months after treatment. At Month 12, cells arising from the progenitor cell account for ~64% to 81% of peripheral blood leukocytes and 80-90% of CD15+ cells. The patient is clinically stable with mild thrombocytopenia due ongoing event of delayed haematopoietic reconstitution.

Relative expression studies could not be performed for *SMG6* expression. For *MECOM*, the results showed elevated expression of Total and Short MECOM transcripts in CD15+ cells in the above subject at Month 65 (when clonal dominance was observed), as compared to a normal human donor. Similarly, in another subject, Total and Short MECOM transcripts were found to be elevated in whole blood at Month 12 as compared to a normal donor control.

The totality of the data collected from transcription analyses of the two predominant clones with IS in *MECOM* is consistent with dysregulation of *MECOM* expression, likely as a result of vector insertion.

Expression dysregulation at the *MECOM* locus could contribute to clonal expansion resulting in clonal predominance, although the mechanism is not clear. *MECOM* expression in both subjects was elevated relative to control cells, however the mechanism of this apparent dysregulation and the relationship to clonal expansion is not known. There is currently no indication that these subjects are overexpressing ALDP as a result.

Both subjects showed a higher clonal distribution in CD15+ cells, suggesting a preference for the myeloid lineage. Data on neutrophilic function assays is not available.

There has been no clinical or haematological evidence of malignancy in any of the subjects to date. Whole exome sequencing was conducted at Month 67 for one Subject (the latter) did not reveal any mutations of interest. Repeated investigation of a selected panel of 95 genes in this subject did not reveal pathogenic variants and cytogenetic analyses results were normal as well. No whole genome or exome sequencing data are available for the former subject to date.

Insertional oncogenesis (e.g. myelodysplasia, leukaemia, lymphoma) has been included in the safety specification of Skysona as an important potential risk.

ISA data will continue to be collected in the ongoing clinical trials, and in the planned registry study, to further the understanding of clonal dynamics in the long term.

A cautionary statement is included in section 4.4 of the SmPC advising that patients be monitored with at least annual peripheral blood counts and additional testing (including bone marrow analysis) if needed.

Safety in special populations

CALD is a rare X-linked disease with paediatric onset, demographics reveal a homogeneous population without sufficient numbers of subjects in different demographic groups to enable meaningful comparisons.

Immunological events

There was no evidence towards a significant risk of hypersensitivity reactions with Skysona, however, study subjects received myeloablative conditioning using busulfan and either cyclophosphamide (Study ALD-102) or fludarabine (Study ALD-104).

In general, the risk of an adaptive immune response to the transgene product or the vector needs to be considered for a gene therapy product. Regarding the vector, since pre-exposition is excluded and no repeated administration is intended, the lack of data is acceptable. The applicant has also sufficiently justified that an assay to detect and measure anti-ALDP antibodies could be omitted as the risk of immunogenicity in terms of anti-ALDP antibody formation is very low.

Safety related to drug-drug interactions and other interactions

No formal drug interaction studies were performed with Skysona. There were no AEs observed in subjects suggestive of any potential drug-drug interaction with Skysona.

Given the theoretical potential for anti-retroviral medications to interfere with transduction of mobilised autologous cells and/or subsequent integration by the LVV, patients should not take anti-retroviral medicinal products from at least one month prior to mobilisation until at least apheresis is completed.

Discontinuation due to AES

There were no AEs leading to withdrawal or discontinuation in Studies ALD-102 and ALD-104, nor in ALD-304 as of the cut-off date. All treated subjects remain on study.

Study ALD-101

Study ALD-101 was a retrospective non-interventional study (data collected April 2011 to March 2012), to characterize the natural history of CALD and identify specific subset(s) of the overall population that would most likely benefit from Skysona. This retrospective chart review study included both patients who received allo-HSCT transplants and those who were untreated.

Only limited comparisons were made with the AE profile of allo-HSCT in this study, as Study ALD-103 included more contemporaneous and comprehensive safety data collection. In summary, the untreated cohort included 72 and the allo-HSCT cohort 65 subjects. During the data collection period, 40/72 (56%) of subjects in the untreated cohort died with underlying disease progression reported as the primary cause of death in 31/40 (78%). The incidence of serious infections in this cohort during this period was 7%. In the allo-HSCT cohort, 16/65 (25%) died during the data collection period with disease progression (7/16, 44%) and infection (5/16, 31%) as the most common causes of death.

The incidence of graft failure was 18% (12/65). Subjects without an HLA-matched donor were most likely to suffer engraftment failure: 8/32 (25%) subjects with mismatched unrelated donors, l/5 (20%) subjects with mismatched related donors.

Despite prophylaxis, the incidence of GVHD was 59%, with acute GYHD in 45% subjects and chronic GVHD in 21% of subjects with the potential to undergo GVHD. The incidence of GVHD was highest amongst those with an HLA-mismatched, unrelated donor (22/27; 82%).

Post marketing experience

N/A.

2.6.2. Discussion on clinical safety

Current safety database

The data supporting the safety profile of Skysona are limited to 45 subjects with CALD that have been treated in single-arm studies, 32 in Study ALD-102 and 13 in Study ALD-104. Limited long-term follow-up data are provided by Study LTF-304.

Treatment with elicel is preceded by haematopoietic stem cell collection (mobilisation with G-CSF and, if needed, plerixafor followed by apheresis and myeloablative conditioning with busulfan and either cyclophosphamide (ALD-102) or fludarabine (ALD-104). The exposure to mobilisation and conditioning agents prior to Skysona infusion should thus be considered in the evaluation of the safety data.

Two completed studies using allo-HSCT were included to provide contextual data. The most contemporary one, Study ALD-103, is a prospective and retrospective data collection study of allo-HSCT and provided safety comparisons for ≥ Grade 3 AEs; ≥Grade 3 AEs related to HSCI; and SAEs. Study ALD-101 is an older, retrospective non-interventional data collection study of subjects either untreated or treated with allo-HSCT.

All subjects treated with Skysona received a single lot with a dose of ≥ 5.0×10^6 CD34+ cells/kg and were followed for a total median (min, max) time of 56.44 (22.1, 70.7) months post-drug product infusion for the initial MAA.

Safety data

In TP-102/104, all subjects followed to at least Rel Day 43 had successful NE, with a median (min, max) NE on Rel Day 13 (11, 41). In TP-103, 53/59 (89.8%) subjects achieved primary NE, with a median (min, max) NE on Rel Day 17 (12, 36). No primary or secondary NE failure was observed in evaluable TP-102/104 subjects. Overall, 43/45 subjects achieved PE by Rel Day 60. One subject achieved PE on Rel Day 108, and one subject is still pending. Both of these subjects developed SAEs of pancytopenia which were still ongoing at the time of the initial data cut. Updated narratives were requested which revealed that the events remain ongoing although neither subject requires transfusions or growth factor support.

Pancytopenia has been included in section 4.4. of the SmPC as well as included in the safety specification as an identified risk.

Primary or secondary NE failure was experienced by 10/38 (26.3%) evaluable subjects by Month 24 in the allo-HSCT study TP-103, all of whom were NMSD subjects. In TP-103, 12 of 59 subjects were not evaluable for PE by Month 24 because they had primary or secondary engraftment failure or died. The 47 evaluable subjects all had successful PE, with median (min, max) PE occurring on Rel Day 26 (13, 67). However, these analyses were based on interim data and 'evaluable subjects' only. Additional and updated analyses that also include all treated patients and time to event methodology were requested. In this analysis, 49 of 51 Skysona treated subjects (96.1%) achieved NE compared to 53 of 59 (89.8%) of allo-HSCT patients. No subjects treated with Skysona experienced either primary or secondary neutrophil engraftment failure (compared to 10/59 (16.9%) subjects treated with allo-HSCT)**.** Most subjects treated with Skysona, 47/51 (92.2%) subjects, achieved PE, compared to 47/59 (79.7%) subjects treated with allo-HSCT. Four subjects in Study ALD-104 had not yet achieved PE or been followed for at least 24 months.

The SmPC has been updated to reflect the analyses based on the entire TP population.

In TP-102/104, almost all subjects experienced AEs that were attributable to mobilisation/apheresis. Frequently reported PTs included hypomagnesaemia, hypokalaemia, nausea, vomiting, anaemia, and bone pain. Also, subjects frequently experienced TEAEs consistent with the effects of conditioning (e.g., thrombocytopenia, anaemia, neutropenia, stomatitis, febrile neutropenia, and alopecia). However, given the close proximity in time of Skysona administration in relation to the conditioning regimen (once the cyclophosphamide regimen was completed, this was followed by 1 rest day before Skysona infusion), some additional AE presentations were requested which did not reveal new concerns.

An assessment of AEs between the ALD-102 and ALD-104 studies shows, in general, a comparable profile considering the small patient population. Some differences were notable (such as gastrointestinal AEs) consistent with the respective agents used. The applicant also considers the NE and PE data between the two regimens to be comparable. This can be partially agreed considering median values but numerically, there seem to be more outlier subjects with later PE in ALD-104; the median (min, max) Rel Day of PE was Rel Day 32 (16, 60) in Study ALD-102 and Rel Day 29 (14, 108) in Study ALD-104. Four of the 19 subjects in the ongoing Study ALD-104 were not yet available for PE as of the latest data cut. However, there were at least 3 subjects with relatively long times to PE) in Study ALD-104 achieved PE after Rel Day 60 (Rel Day 104, 108, and 106 respectively). In addition, two of these subjects were reported with SAEs of pancytopenia (still ongoing). Thus, relevant safety information obtained with ALD-104 is included in the SmPC. However, given the absence of sufficient efficacy data and the limited safety data available, a full B/R evaluation on this conditioning regimen is not yet possible.

A total of 5/45 (11.1%) subjects had TEAEs that were considered by the Investigator to be drug product related TEAEs. These included Grade 1 events of vomiting likely related to DMSO used as a cryopreservative in Skysona drug product; one severe case of haemorrhagic cystitis, and the above-stated two cases of ongoing pancytopenia. Post-engraftment cytopenias that required growth factor treatment were reported and 30 of 45 subjects in TP-102/104 received G-CSF in the period after NE. In most of these subjects, G-CSF was administered continuously from prior to NE to shortly after NE, was brief in duration and often associated with a clinical finding (e.g., febrile neutropenia). This with the exception of the 2 subjects who experienced delayed haematopoietic reconstitution and who received G-CSF for more than 100 days after the subjects achieved NE. These 2 subjects also received eltrombopag due to decreased platelets.

All 45 patients were treated with antibiotic during the conditioning therapy (thus C to <NE). The majority (39, 86.7%) were treated with antibiotic due to an adverse event however, in the majority (n=35) of these patients, the adverse event was not directly related to an infection but rather a symptomatic treatment in cases where infections could not be excluded (due to e.g. 'Pyrexia', or 'Febrile neutropenia').

Section 4.4 of the SmPC has been updated to include a warning for prolonged cytopenia and that cases of pancytopenia have been reported, and these events are included in the safety specification as an important identified risk. In addition, delayed platelet engraftment has been included in the safety specification as a potential risk.

A contextual comparison for AEs between TP-102/104 and TP-103 could only be made for≥ Grade 3 TEAEs through Month 12 (excluding haematological toxicities related to conditioning medication), as well as ≥ Grade 3 TEAEs related to HSCT and TESAEs through Month 48. The pattern of Grade 3 non-haematological AEs that were attributed to conditioning by the Investigator was generally similar between TP-103 (36/59 [61%]) and TP-102/104 (29/45 [64.4%]). A higher percentage of subjects experienced ≥ Grade 3 TEAEs of infection attributed to conditioning in TP-103 (16/59 [27.1%]) as compared to TP-102/104 (2/45 [4.4%]) which may be related to differences in the respective conditioning regimens used or difference in baseline characteristics. None of the infections except for the case of viral cystitis was considered related to Skysona by the investigator. In Study TP-103, there were 4 fatal infections experienced by 3 subjects (see deaths below).

No evaluable subjects (0/31) in TP-102 experienced either acute (≥ Grade II) or chronic GVHD by Month 24, compared to 26/50 (52%) evaluable subjects in TP-103. However, as these analyses are based on 'evaluable subjects' only, some clarifications and supplementary analyses were requested and the SmPC was updated to reflect the analyses based on the entire TP population. Time to first acute (≥Grade II) or chronic GVHD event analyses were also performed to supplement the proportional primary endpoint, in line with scientific advice (EMA/CHMP/SAWP/82607/2018).

One death (1/45, 2.2%) was reported in TP-102/104 and a second subject died after receiving allo-HSCT off study. None of the deaths was considered related to Skysona. In comparison, 15/59 (25.4%) subjects died in TP-103 (12 after first allo-HSCT and 3 after second allo-HSCT), 9 of whom had transplant-related mortality (TRM).

Overall, 27/45 (60%) subjects experienced 63 TESAEs from D1 through Last Follow-up. The overall SAE profile was largely consistent with the effects of conditioning. The applicant provided an updated narrative for a reported case of transverse myelitis, together with a discussion whether Skysona could have triggered an immune reaction but this appears to be unlikely.

Limited long-term safety data were presented for 21 subjects enrolled in the ongoing open-label extension study LTF-304. As of the initial cut-off date, no subjects experienced GVHD or underwent a

second stem cell transplantation. Seven SAEs in 4 subjects were reported, none of which were attributed to Skysona.

There were 5 subjects who reported new TESAEs as of the new data cut (Q4 2020). In addition, the applicant reported 5 new (additional) SAEs as Late Breaking Information. Overall, 6 of these new SAEs related to serious events of infection/pyrexia/neutropenia and 4 events related to seizures. Adding these late-breaking events, the proportion of subjects with SAEs of infection seems to be similar for both the ALD-102 and ALD-104 studies, i.e. numerically not favouring the new conditioning regimen. However, the numbers are small, and this would need to be further followed.

At the time of the interim data cut, there were no other AEs or events of interest attributable to the use of an LVV for the ex vivo transduction of autologous cells, i.e. no detection of replication of competent lentivirus (RCL), or malignancy.

ISA analyses in the 32 treated subjects from ALD-102 with available ISA results as of 04 February 2020 (up to 6 years of follow-up) showed 334,492 unique IS being detected in 191 samples. Some gene locations were detected amongst the Top 10 IS per subject per visit at multiple times both between and within subjects, with IS in the *SMG6* and *MECOM* genes being the most frequently detected.

One subject has shown a persistent high Relative IS-Frequency of 3 IS (with one of the IS in the *MECOM* gene) in a single clone over time. Since the initial MAA, one additional subject from Study ALD-104 has also been identified with a predominant clone with 4 IS, including an IS in *MECOM*.

In a late-breaking update (31 March 2021), the applicant has provided updated information from the Year 6 evaluation (January 2021) for one of these subjects. ISA results confirmed the persistence of the predominant clone previously identified at Month 65. Analysis of subpopulations of blood cells showed that this clone was most highly represented in cells of the myeloid lineage, increasing in its clonal contribution to CD15+ cells from approximately 21% to 25% at Month 48 to 74% to 81% at Year 6. As this clone thus exceeded a clonal contribution to CD15+ cells of 50% at both Month 65 and Year 6 Visits, it satisfied the protocol definition of being a persistent predominant clone in this cell population.

The subject's clinical status is unchanged with normal haemoglobin, platelets, and white blood cell count and a neutrophil count at the lower end of the normal range and will continue to be monitored closely.

Relative expression studies showed elevated expression of Total and Short MECOM transcripts in CD15+ cells in this subject at Month 65 (when clonal dominance was observed), as compared to a normal human donor. Similarly, in another subject, Total and Short MECOM transcripts were found to be elevated in whole blood at Month 12 as compared to a normal donor control.

There is currently no indication that these subjects are overexpressing ALDP as a result.

The applicant concludes that these findings are consistent with dysregulation of *MECOM* expression, likely as a result of the insertion of the LVV into the *MECOM* gene. The mechanism for the increased *MECOM* expression is unclear. While the applicant is suggesting that is unlikely that the MNDU3 promoter is directly controlling MECOM expression, this is the most plausible explanation in line what has previously been reported for X-CGD and Wiskott Aldrich clinical trials. The position of the integration of the two dominant clones in these subjects is in the same region of MECOM (intron 2) of dominant IS in patients with X-CGD and Wiskott-Aldrich Syndrome that eventually developed AML after 2-3 years from the initial clonal dominance. Internal viral promoters have been described in preclinical models to influence expression of nearby genes even when placed internally in self-

inactivating vectors. On the other hand, X-CGD and Wiskott-Aldrich Syndrome may carry additional intrinsic risk factors as compared to X-ALD cells.

Thus, it is possible that these dominant clones will remain benign, such as in most cases of clonal haematopoiesis in aging, without evolving in leukaemia. There is no clinical or haematological evidence of malignancy to date.

Cases of haematologic malignancies due to insertional oncogenesis following gene therapy have been reported to occur with retroviral vectors but not the modified LVV to date. The potential risk of insertional oncogenesis will need to be handled in the RMP, however.

The potential need for additional post-authorisation follow-up for Skysona has been previously discussed with the applicant, as outlined in e.g. various scientific advice procedures. In addition to the ongoing study LTF-304, the applicant proposes to conduct the post-approval registry study REG-502 (see also Section 3.4.9 below).

Given that the ISA analyses form an area of significant safety interest, the applicant will continue to collect ISA data in the setting of studies and evaluate its association with clinical events. In study LTF-304, ISA is to be scheduled every 6 months for 5 years and then annually to Year 15. In the planned study REG-502, ISA is also offered on an annual basis until the end of the study.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Overall, the current safety data are considered favourable in the context of the intended population. Additional safety data will be required for the long-term follow-up of the product in accordance with risks described in the safety specification. In addition, the additional safety data from the ongoing ALD-104 study will be of value to further inform the prescriber on the safety of the product in combination with other conditioning regimens than the one used in the pivotal ALD-102 study.

2.6.3. Conclusions on clinical safety

Overall, based on the current limited data, the safety profile of Skysona appears to be acceptable; however, it should be considered that, similar to allo-HSCT, treatment with Skysona is preceded by significant procedural and medical interventions, including haematopoietic stem cell collection and myeloablative conditioning. The safety of Skysona seems clearly favourable over allo-HSCT for those subjects without a matched sibling donor. There are outstanding uncertainties related to long-term safety, including the potential risk of insertional oncogenesis, since two patients have been shown to have a dominant clone, albeit without clinical symptoms.

In addition to the ongoing study LTF-304, the applicant proposes to conduct the post-approval registry study REG-502. Additional safety data will indeed be required for the long-term follow-up of the product in accordance with risks described in the safety specification. In addition, the additional safety data from the ongoing ALD-104 study will be of value to further inform the prescriber on the safety of the product in combination with other conditioning regimens than the one used in the pivotal ALD-102 study.

Overall, the safety profile of Skysona is considered favourable for a positive Benefit/Risk balance from a safety point of view considering the intended patient population. The CAT considered the following measures necessary to further inform the long-term safety profile of the product:

- In order to further characterise and contextualise the long-term safety and efficacy of Skysona in patients with cerebral adrenoleukodystrophy (CALD), the MAH should conduct, and submit the results of a prospective observational Registry Study (REG-502) of patients with CALD treated with Skysona or

allogeneic haematopoietic stem cell transplantation (allo-HSCT) according to an agreed protocol (Stargazer).

- In order to evaluate the long-term efficacy and safety of Skysona in patients with cerebral adrenoleukodystrophy (CALD), the MAH should submit final results of Study LTF-304.

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

2.7. Risk Management Plan

Safety concerns

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Prolonged cytopenias/ pancytopenia	**Routine risk minimisation measures** – SmPC sections 4.4 and 4.8 – PL sections 2 and 4 Monitoring of blood counts and evaluation of patients for signs and symptoms of bleeding and infection in SmPC section 4.4. Restricted prescription medicine **Additional risk minimisation measures** – Educational materials for healthcare professionals – Educational materials for patients/parents/carers – Patient alert card	**Additional pharmacovigilance** – REG-502 (final CSR: Q2 2042) – LTF-304 (final CSR: Q2 2037) – ALD-102 (final CSR: November 2021) – ALD-104 (final CSR: Q2 2024)
Insertional oncogenesis (e.g. myelodysplasia, leukaemia, lymphoma)	**Routine risk minimisation measures** – SmPC sections 4.4 and 5.3 – PL section 2 At least annual monitoring for myelodysplasia, leukaemia, or lymphoma (including a complete blood count) for 15 years post treatment with Skysona is recommended in SmPC section 4.4 and PL section 2. If myelodysplasia, leukaemia, or lymphoma is detected, collection of blood samples for integration site analysis is recommended in SmPC section 4.4. Restricted prescription medicine **Additional risk minimisation measures** – Educational materials for healthcare professionals – Educational materials for patients/parents/carers – Patient alert card	**Additional pharmacovigilance** – REG-502 (final CSR: Q2 2042 :) – LTF-304 (final CSR: Q2 2037) – ALD-102 (final CSR: November 2021) – ALD-104 (final CSR: Q2 2024)

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Lack or loss of response to gene therapy	**Routine risk minimisation measures** Restricted prescription medicine **Additional risk minimisation measures** – Educational materials for healthcare professionals – Educational materials for patients/parents/carers	**Additional pharmacovigilance** – REG-502 (final CSR: Q2 2042) – LTF-304 (final CSR: Q2 2037) – ALD-102 (final CSR: November 2021) – ALD-104 (final CSR: Q2 2024)
Neutrophil engraftment failure	**Routine risk minimisation measures** – SmPC sections 4.2 and 4.4 – PL section 2 Restricted prescription medicine **Additional risk minimisation measures** – Educational materials for healthcare professionals	**Additional pharmacovigilance** – REG-502 (final CSR: Q2 2042CSR) – ALD-102 (final CSR: November 2021) – ALD-104 (final CSR: Q2 2024)
Platelet engraftment failure	**Routine risk minimisation measures** Restricted prescription medicine **Additional risk minimisation measures** – Educational materials for healthcare professionals – Educational materials for patients/parents/carers	**Additional pharmacovigilance** – REG-502 (final CSR: Q2 2042) – ALD-102 (final CSR: November 2021) – ALD-104 (final CSR: Q2 2024)
Long-term safety and efficacy	**Routine risk minimisation measures** – SmPC section 5.1 Restricted prescription medicine **Additional risk minimisation measures** – None	**Additional pharmacovigilance** – REG-502 (final CSR: Q2 2042) – LTF-304 (final CSR: Q2 2037) – ALD-102 (final CSR: November 2021) – ALD-104 (final CSR: Q2 2024)

Abbreviations: CSR = clinical study report; PL = package leaflet; Q = quarter; SmPC = summary of product characteristics.

Conclusion

The CHMP, CAT and PRAC considered that the risk management plan version 0.4 is acceptable.

2.8. 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 CAT/CHMP Opinion. The IBD will be the EBD. The new EURD list entry will therefore use the European Birth Date (EBD) to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant declared that elivaldogene autotemcel has not been previously authorised in a medicinal product in the European Union.

The CAT/CHMP, based on the available data, considers elivaldogene autotemcel to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. 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.*

2.10.2. 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; the use of minimum particulars for the bag infusion label was agreed.

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:

The Group accepted the exemption requests to use English only for the labelling components/package leaflet. Furthermore, the Group also agreed with the provision of the use of the national languages for the Lot Information Sheet and for the package leaflet at the time of consent to treatment.

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.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Skysona (elivaldogene autotemcel) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, 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

The claimed therapeutic indication is: *Skysona is indicated for the treatment of early cerebral adrenoleukodystrophy in patients less than 18 years of age, with an ABCD1 genetic mutation, and for whom a human leukocyte antigen (HLA)-matched sibling haematopoietic stem cell (HSC) donor is not available (see section 5.1).*

Adrenoleukodystrophy (ALD) is a rare, X-linked, metabolic disease in which dysfunction or lack of the ALD protein (ALDP) is caused by mutations in the ATP-binding cassette, subfamily D member 1 (*ABCD1*) gene. ALDP is a peroxisomal transport protein involved in the transport and degradation of very long chain fatty acids (VLCFA), leading to accumulation of VLCFAs, which occurs most prominently in the adrenal cortex and white matter of the brain and spinal cord. Cerebral ALD (CALD) is the most severe form of ALD, affecting approximately 30-40% of boys with ALD, typically during childhood. CALD is characterised by inflammation of white matter and rapidly progressive cerebral demyelination leading to progressive, irreversible loss of neurologic function. Disease progression and neurologic decline in patients with CALD have been documented in natural history studies; patients experience critical deficits across multiple neurological domains within a short period after onset of clinical symptoms (Suzuki et al. 2005). In children diagnosed with CALD, learning and behavioural problems are often observed at the time of disease onset in early- to mid-childhood (median age 7 years) with progressive gait, vision, and hearing impairments within 6 to 15 months of symptom onset (Suzuki et al. 2005). This is typically followed by rapid neurologic functional decline (Moser et al. 2007) (CSR ALD-101). If untreated, nearly half of patients with CALD die within 5 years of symptom onset.

For boys with a genetic diagnosis of ALD, regular MRIs of the brain are used to detect early signs of onset of CALD (i.e. contrast enhancement, white matter involvement) which indicate that a patient is at high risk for rapid progression. When signs of CALD have developed, the patient should be treated. The aim of allo-HSCT or Skysona, which is an autologous HSCT with ex vivo lentiviral vector gene transfer of the *ABCD1* gene, is to halt neuroinflammation and cerebral demyelination and maintain independent living for the affected boys. No effects of allo-HSCT have been seen on other disease manifestations of ALD, such as adrenal insufficiency, and the slowly developing spinal and peripheral nerves neuropathy in adulthood. It is anticipated that Skysona will only have beneficial effects on the cerebral inflammation part of the disease, just as has been observed for allo-HSCT treatment.

Males who do not develop childhood cerebral ALD may still develop more benign variants of the disease, the most common being a more slowly progressive adrenomyeloneuropathy (AMN) which typically develops during the 2nd to 3rd decade of life. A few adult males may develop the cerebral form of the disease, but with different disease characteristics (including psychiatric symptoms) than the childhood CALD. Furthermore, in adults, the cerebral symptoms are often superimposed on the myeloneuropathy. Women are normally only carriers of the *ABCD1* gene mutation and will transfer the mutation to their offspring. However, women may develop mild AMN in adult age and there are a few case reports of girls with childhood CALD.

3.1.2. Available therapies and unmet medical need

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is a therapeutic option that has been used for a few decades and that can stabilise progression of CALD. The best outcomes are observed if it is performed at early stages of cerebral involvement, i.e. MRI scoring Loes ≤9 and Neurological

Function Scale (NFS) ≤1. Allo-HSCT has potential complications, including risks for transplant-related mortality, graft failure, graft-versus-host disease, and infection. These risks are decreased in patients who have a human leukocyte antigen-matched sibling donor; which fewer than 30% of patients have. There is a high unmet medical need for efficacious therapies for CALD that have an improved safety profile compared to allo-HSCT, especially for those who do not have a matched sibling donor.

The unmet medical need for patients with CALD has been acknowledged in the applicant's request for accelerated assessment that had been granted.

3.1.3. Main clinical studies

The main evidence of efficacy stems from study ADL 102. Data from the ongoing study ALD-104 and long term follow up ALD-304 support the safety and efficacy of the product. Studies ALD-101 and ALD-103 are historical control studies describing disease progression and neurological decline in untreated CALD patients or allo-HSCT treated patients.

The ongoing pivotal open-label, single-arm, multi-site study **ALD-102** enrolled male subjects ≤17 years with 'early CALD' (defined as having contrast enhancement on brain MRI (GdE+), the MRI score Loes 0.5-9 and NFS ≤1) and without an available matched sibling donor. This study used busulfan and cyclophosphamide as conditioning treatment. The study population is divided into the Initial cohort (N=17), mainly used for the primary endpoint and success criterion calculation, and the Overall cohort with all enrolled subjects (N=32), used in all other endpoints. At the time of data cut-off for study ALD-102 (17 Jan 2020), 23 subjects in the overall cohort had reached the month 24 end of study or had withdrawn from the study. The primary endpoint of the main studies was proportion of subjects that were alive and free from major functional disabilities (MDF) at 24 months (MFD-free survival), with the 6 major functional disabilities defined as complete loss of volunteer movement, wheelchair dependence, tube feeding, total incontinence, cortical blindness, and loss of communication. In addition to experiencing any MFDs or death, the following events were also considered as a failure to meet the primary efficacy endpoint: requirement for rescue cell administration or an allo-HSCT, withdrawal from study, or lost to follow-up by Month 24. Secondary endpoints included overall survival, NFS, Loes score, and being free from contrast enhancement (GdE-) at Month 24. Exploratory endpoints included neuropsychological tests, Pediatric Quality of Life Inventory (PedsQL) score, Global Assessment Scores, and pharmacodynamic endpoints: Vector copy number (VCN) in peripheral blood cells, Percent of peripheral blood cells expressing ALDP, change from Baseline to Month 24 in VLCFA levels in fasting serum.

The ongoing supportive open-label, single-arm, multi-site study **ALD-104** enrolled male subjects ≤17 years with early CALD (defined as GdE+, Loes ≤9 and NFS ≤1) and without an available matched sibling donor. This study used busulfan and fludarabine (instead of cyclophosphamide) as conditioning treatment. At the time of data cut-off for MAA submission, 13 subjects had been treated, but none had reached the month 24 end of study. No efficacy results are presented from this study at this stage, only PD data and safety results. The endpoints were the same for this study as for ALD-102.

The ongoing supportive non-interventional long-term follow-up study **LTF-304** enrols all subjects that have concluded their participation in the two main studies (ALD-102 and 104) and continues to follow these subjects up to 15 years after Skysona treatment. At the time of data cut-off, 21 patients from study ALD-102 had enrolled in the LTF-304 study, with a median (min, max) duration of follow-up of 56.44 (22.1, 70.7) months after drug product infusion. This study provides some maintenance of efficacy results and long-term safety.

The concurrent control study **ALD-103** was a prospective and retrospective data collection study to evaluate outcomes in males ≤17 years treated with allogeneic haematopoietic stem cell transplantation

(allo-HSCT) for CALD. The study included in total 59 subjects. The subpopulation (N=27) TPES (Strictly ALD-102-Eligible Transplant Population) fulfilled the inclusion criteria from studies ALD-102 and ALD-104 (defined as GdE+, Loes ≤9 and NFS ≤1) and was used for comparison with Skysona treatment. From the TPES subgroup, two subgroups were defined based on donor type: subjects with a matched sibling donor (MSD, N=10), or without a matched sibling donor (NMSD, N=17).

The retrospective, multi-centre historical control study **ALD-101** collected data on natural course of disease in untreated patients or effects of allo-HSCT from boys 3-15 years old with a diagnosis of symptomatic CALD and a Loes score of >0 and <15 and at least 2 years follow-up. The study included 72 untreated subjects and 65 subjects who received allo-HSCT. These results were used for obtaining benchmarks for Skysona, in particular to provide survival data from an untreated cohort.

3.2. Favourable effects

In the pivotal study ALD-102, the proportion of patients alive and free from loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, complete loss of voluntary movement (24 MFD-free survival) was 15/17 (88.2%, 95% CI: 63.6, 98.5) in the Initial cohort and 20/23 (87.0%, 95% CI: 66.4, 97.2) in the Overall cohort. The 'success criterion' was based upon having a lower 95% CI of >50.0% for the endpoint of Month 24 MFD-free survival for the Initial Cohort (N = 17). This was based on comparison of the primary efficacy endpoint results to a clinically meaningful benchmark (study ALD-101 untreated cohort and published data). Since the lower 95% CI was above 50% (63.6%) for the initial cohort, the 'success criterion' for efficacy was met. In study ALD-101, only 1 patient in the untreated control cohort fulfilled the eligibility criteria in the ALD-102 study (i.e. GdE+, Loes 0.5-9, NFS≤1), which prevented comparisons with an exactly matching untreated subgroup. However, other subgroups fulfilling the criteria partially confirm that the 2-year (MFD-free) survival is low in the untreated CALD patient. As an example, the untreated cohort in study ALD-101 who were GdE+ (N=21) had a 2-year MFD-free survival of 29% from the first GdE+ brain MRI scan. In study ALD-101, the overall 5-year survival from CALD diagnosis was 55% in the overall (N=72) untreated cohort. Based on the long-term experience from allo-HSCT treatment of CALD, being the current standard of care, these limitations of comparability with an untreated cohort are acceptable and the short- and mid-term benefit of Skysona treatment over no treatment is considered established.

In study ALD-103, the allo-HSCT MSD (matched sibling donor) subgroup (N=9) had an 88.9% (95% CI:51.8, 99.7) Month 24 MFD-free survival, whereas the subgroup without a matched sibling donor (NMSD, N=9) had 66.7% (95% CI: 29.9, 92.5) Month 24 MFD-free survival.

The proportion of GdE- MRI scans at Month 24 was 81% in 21 evaluable subjects (95% CI: 58.1, 94.6) in study ALD-102. In the study ALD-103 TPES population, the corresponding proportion was 100% (95%CI: 75.3, 100.0) among the 13 evaluable subjects.

The proportion of subjects experiencing a stable NFS (NFS <=4 without an increase of >3 points from Baseline) at month 24 was 95.7% (95% CI: 78.1, 99.9) in 23 evaluable subjects in study ALD-102. In the study ALD-103 TPES population, the corresponding proportion was 100% (95%CI: 73.5, 100.0) among the 12 evaluable subjects.

The proportion of subjects experiencing a stable Loes score (≤9 or not increased by ≥6 points from Baseline) at month 24 was 76.2% (95% CI: 52.8, 91.8) in 21 evaluable subjects in study ALD-102. In the study ALD-103 TPES population, the corresponding proportion was 92.3% (95% CI: 64.0, 99.8) in 13 evaluable subjects. The few (n=4) subjects with a baseline Loes score ≥ 4.5 had a greater worsening of Loes score (+7.5, vs +1.0) and Performance IQ (-23 vs -2.5) from baseline to month 24 than subjects with a baseline Loes score of <4.5 (n=28).

Performance IQ for all subjects in ALD-102 show that most subjects remained within the normal IQ range of 100 ± 15 points (i.e. 85-115 points). A few subjects (n=2) had a baseline IQ below 70 and stayed below 70 after treatment. A few (n=2) started at a baseline IQ of above 70 and decreased to below 70 after treatment.

In peripheral blood, PK/PD parameters including VCN, ALDP+ cells and reductions of VLCFAs are persisting over longer time follow up, although reductions are seen over time. The median % change in the VLCFA C26:0 LysoPC from baseline to Month 24 was -20% (min, max: -59, +94) in 20 evaluable subjects in study ALD-102. In the study ALD-103 TPES population, the corresponding % change was -61% (min, max: -75, +5), albeit as measured in only 5 evaluable subjects. The reductions of VLCFA seen after Skysona and allo-HSCT treatment do not normalise VLCFAs to normal values seen in healthy men, but rather towards the range of female carriers.

From the ongoing long-term follow-up study LTF-304, efficacy data from the first 21 subjects enrolled showed that efficacy was generally maintained during the reported follow-up period of 56.44 (22.1, 70.7) months. During their follow-up in LTF-304, none of the 21 subjects had developed MFDs or died. In study LTF-304, 3 subjects had a resolution of their GdE+ whereas 1 subject developed GdE+, 3 subjects had increased their NFS by 1 point, and Loes score was generally stable over time beyond Month 24. In comparison, MFD-free survival was further decreased in subjects treated with allo-HSCT in study ALD-103, in which it stabilised only from Month 36. Thus, the Kaplan-Meier estimated Month 36 MFD-free survival was 90.3% (95% CI: 72.9, 96.8) in ALD-102/LTF-304 vs 74.1% (28.9, 93.0) for the TPES-103 MSD subgroup and 58.8% (27.5, 80.4) for the TPES-103 NMSD subgroup. The worse MFD-free survival at month 36 in the allo-HSCT subgroups were related to immuno-incompatibility between donor and recipient, i.e. GvHD and second allo-HSCT due to graft rejection, and not to disease progression.

From the latest data cut-off for the MAA, 51 subjects have been followed for a median (min, max) 29.11 (0.1, 82.7) months and a total of 137.1 patient years. In studies ALD-102/LTF-304, the median (min, max) duration of follow-up was 38.59 (13.4, 82.7) months. Nearly half of subjects (20/51) have been followed for at least 3 years, with 11 subjects followed for at least 5 years. In study ALD-104, the median (min, max) duration of follow-up was 8.64 (0.1, 16.8) months and only 8 subjects had completed their Month 12 visits.

3.3. Uncertainties and limitations about favourable effects

The methodological limitations that arise from the design of the studies are associated with general difficulties to design and conduct studies in rare diseases with limited patient populations, with few or inadequate treatment options as is the case with CALD. These limitations, in terms of small sample sizes, no concurrent comparator arm, and use of external controls, were all acknowledged during the PRIME development discussions. Limitations of the study designs allow the interpretation of results for descriptive purpose only. Selection of endpoints presented in the SmPC section 5.1 is not based on statistical grounds but on the clinical relevance of the obtained results.

The maintenance of efficacy over longer time than 60-80 months is currently unknown. This will be followed in the two long-term follow-up studies LTF-304 and REG-502, which are considered key to the Benefit/Risk ratio of the medicinal product.

The efficacy of Skysona after the alternative conditioning treatment of busulfan and fludarabine as in study ALD-104 is currently also unknown, since this study is ongoing and the timepoint for the primary endpoint MFD-free survival has not been reached yet for any of the patients. Further data are being collected to address this in studies ALD-104 and LTF-304.

3.4. Unfavourable effects

The Transplant Population (TP) consisted of subjects who received Skysona infusion or allo-HSCT in each study.

In TP-102/104, all subjects followed to at least Rel Day 43 (with Day 1 defined as the day of drug product infusion) had successful neutrophil engraftment (NE), with a median (min, max) NE on Rel Day 13 (11, 41). In TP-103, 53/59 (89.8%) subjects achieved primary NE, with a median (min, max) NE on Rel Day 17 (12, 36). No primary or secondary NE failure was observed in evaluable TP-102/104 subjects. Overall, 43/45 subjects achieved platelet engraftment (PE) by Rel Day 60.

In comparison, primary or secondary NE failure was experienced by 10/38 (26.3%) evaluable subjects by Month 24 in the allo-HSCT study TP-103, all of whom were NMSD subjects. In TP-103, 12 of 59 subjects were not evaluable for PE by Month 24 because they had primary or secondary engraftment failure or died. The 47 evaluable subjects all had successful PE, with median (min, max) PE occurring on Rel Day 26 (13, 67).

Updated analyses that also included all treated patients were consistent and showed that 49 of 51 Skysona treated subjects (96.1%) achieved NE compared to 89.8% in TP-103; 2 subjects from Study ALD-104 had not yet achieved NE or been followed to at least Rel Day 43. Most subjects treated with eli-cel, 47/51 (92.2%) subjects, achieved PE, compared to 47/59 (79.7%) subjects treated with allo-HSCT. Four subjects in Study ALD-104 had not yet achieved PE or been followed for at least 24 months.

Two subjects in Study ALD-104 had ongoing SAEs of pancytopenia but were clinically stable with no further need for platelet supportive treatments.

Updated results for clinical laboratory evaluations showed severe depletion of neutrophils and platelets during and after conditioning with busulfan and cyclophosphamide (ALD-102) and busulfan and fludarabine (ALD-104), respectively, in particular, as intended, initially. During the >M12 to M24 period, 4/38 (10.5%) subjects experienced a potentially clinically significant (PCS) low leukocyte result (<4.0 x 10^9/L), 3/38 (7.9%) subjects experienced a potentially clinically significant PCS low neutrophil result (<1.0 x 10^9/L), and 1/38 (2.6%) subject experienced a PCS low platelet result ≤75 x10^9/L). After Rel Day 100, 15.6% of subjects had any Grade 3 or higher cytopenia, including decreased platelet count (8.9%), decreased neutrophil count (11.1%), and decreased haemoglobin (0%). Prolonged Cytopenias' has been characterised as an Important Identified Risk and updates to section 4.4 of the SmPC have been added.

In TP-102/104, almost all subjects experienced TEAEs that were attributable to mobilisation/apheresis. Frequently reported PTs included hypomagnesaemia, hypokalaemia, nausea, vomiting, anaemia, and bone pain. Also, subjects frequently experienced TEAEs consistent with the effects of conditioning.

A similar TEAE pattern was observed in TP-103. However, a higher percentage of subjects experienced ≥ Grade 3 TEAEs of infection attributed to conditioning in TP-103 (16/59 [27.1%]) as compared to TP-102/104 (2/45 [4.4%]).

No evaluable subjects (0/31) in TP-102 experienced either acute (≥ Grade II) or chronic GVHD by Month 24, compared to 26/50 (52%) subjects in TP-103, 3/11 (30%) in the MSD group and 23/48 (57.5%) in the NMSD group. An updated analysis for the entire TP showed no subjects treated with Skysona (TP-102/104) experienced either acute (≥ Grade II) or chronic GVHD as compared to 26/59 (44.1%) subjects who received allo-HSCT in TP-103. For TPES-103 the percentage was 10/27 (37.0%) accordingly.

There were differences in the TESAE profiles of TP-102/104 and TP-103 illustrative of the need for post-transplant immunosuppression in allo-HSCT recipients vs autologous eli-cel recipients.

The proportion of subjects with serious infections was more than 2-fold higher in TP-103 than in TP-102/104 (22/59 [37.3%] vs 7/45 [15.6%], respectively), and the number of serious infection events was more than 5-fold higher (47 events vs 8 events, respectively). Many serious infections in TP-103 were opportunistic (14 SAEs), reflective of the subjects' chronically immunocompromised state, while 1 serious opportunistic infection was reported in TP-102/104 (Cystitis viral). The majority of TESAE infections (38/47 events) in TP-103 were reported in 16 subjects in the NMSD subgroup.

There were no TESAEs reported in the Vascular disorders SOC for TP-102/104. However, 7/59 (11.9%) subjects in TP-103 experienced 8 TESAEs in this SOC, with 6 of these 7 subjects in the NMSD subgroup. Two subjects had serious events of hypertension, a known consequence of immunosuppressants, and 4 subjects had 4 events that were thrombotic (e.g., cerebral infarction, deep vein thrombosis, thrombosis, and veno-occlusive disease).

There were no TESAEs related to autoimmunity in TP-102/104; there was one reported ongoing SAE of transverse myelitis in Study ALD-104 but a causal relation to elicel was considered unlikely.

2 subjects in TP-103 experienced 3 TESAEs attributed to autoimmunity by the Investigator. One NMSD subject experienced a TESAE of Autoimmune haemolytic anaemia and an MSD subject experienced 2 TESAEs attributed to autoimmunity by the Investigator, Haemolytic anaemia and encephalopathy.

One death (1/45, 2.2%) was reported in TP-102/104 and a second subject died after receiving allo-HSCT off study. None of the deaths was considered related to eli-cel.

In comparison, 15/59 (25.4%) subjects died in TP-103 (12 after first allo-HSCT and 3 after second allo-HSCT), 9 of whom had transplant-related mortality (TRM). Thirteen of the 15 deaths were reported in NMSD subjects.

As of the updated Q4 2020 cut-off date, no subjects experienced GVHD or underwent a second stem cell transplantation. Seven SAEs in 4 subjects were reported in the initial MAA, none of which were attributed to eli-cel. There were 5 subjects who reported new TESAEs as of the new data cut. In addition, the applicant reported 5 new (additional) SAEs as Late Breaking Information. Overall, 6 of these new SAEs related to serious events of infection/pyrexia/neutropenia and 4 events related to seizures. Adding these late-breaking events, the proportion of subjects with SAEs of infection seems to be similar for both the ALD-102 and ALD-104 studies.

3.5. Uncertainties and limitations about unfavourable effects

Safety data from the ongoing study ALD-104, which utilised a different conditioning regimen, were particularly limited at this stage and while the available data indicate comparable findings it is therefore not yet known whether the use of different lymphodepleting agents (cyclophosphamide vs. fludarabine) is an important extrinsic factor in eli-cel treated subject safety.

Long-term safety data are still very limited. At the time of the interim data cut, there were no AEs of interest attributable to the use of an LVV for the *ex vivo* transduction of autologous cells, i.e. no detection of replication of competent lentivirus (RCL), or malignancy. However, one subject (2011) in the ongoing Study LTF-304 who received eli-cel in Study ALD-102 and one subject (2208) in Study ALD-104 have developed a predominant clone. The two genes most frequently identified with IS in the study population are *SMG6* and *MECOM* and both subjects had an IS in the *MECOM* gene. Transcription analysis studies indicate increased MECOM expression, the mechanism of which and possible clinical significance in terms of risk for malignancy are currently unclear.

Recently provided (31 March 2021) updated information on Subject 2011 confirmed persistence of this predominant clone at the Year 6 evaluation for this subject (January 2021). The subject is clinically stable, with normal haemoglobin, platelets, and white blood cell count and his previous mild neutropenia has resolved.

Cases of haematologic malignancies due to insertional oncogenesis following gene therapy have been reported to occur with retroviral vectors but not the modified LVV to date.

3.6. Effects Table

Table 34 Effects Table for Skysona for the treatment of CALD in children (data cut-off for study ALD-102: 17 Jan 2020)

Effect	Short Description	Unit	Treatment (eli-cel)	Control Allo-HSCT (MSD, N=10)	Control Allo-HSCT (NMSD, N=17)	Uncertainties/ Strength of evidence	References
	Favourable Effects						
Month 24 MFD-free survival	No MFD, alive, not withdrawn or lost to FU, no rescue eli-cel, no allo-HSCT	% (95% CI)	Initial cohort (N=17) 88.2% (63.6, 98.5) Overall cohort (N=23) 87.0% (66.4, 97.2)	N=9 88.9% (51.8, 99.7)	N=9 66.7% (29.9, 92.5)	Comparison between two separate studies, not all subjects included in the analysis	ALD-102 ALD-103 TPES
Month 24 GdE-	resolution of gadolinium positivity at month 24	% (95% CI)	(N=21) 81% (58.1, 94.6)	(N=13) 100% (75.3, 100.0)		See above	ALD-102 ALD-103 TPES
*Stable NFS at month 24	neurological function scale (0-25)	% (95% CI)	(N=23) 95.7% (78.1, 99.9)	(N=12) 100% (73.5, 100.0)		See above	ALD-102 ALD-103 TPES
¤Stable Loes score at month 24	Brain MRI severity score (0-34)	% (95% CI)	(N=21) 76.2% (52.8, 91.8)	(N=13) 92.3% (64.0, 99.8)		See above	ALD-102 ALD-103 TPES
Change in VLCFA at Month 24	Change in C26:0 LysoPC	% median (min, max)	(N=20) -20% (-59, +94)	(N=5) -61% (-75, +5)		See above	ALD-102 ALD-103 TPES
	Unfavourable Effects						
Serious infections	D1 to last FU	n (%)	7/45 (15.6%)	6/11 (54.5%)	16/48 (33.3%)	See above	ALD-102 ALD-103
Death	D1 to last FU	n (%)	1/45 (2.2%)	2/11 (18.2%)	10/48 (20.8%)	See above	ALD-102 ALD-103
GHVD	Acute (≥ Grade II) or chronic GVHD by Month 24	n %	0/31 (0)	3/10 (30%)	23/40 (57.5%)	See above	ALD-102 ALD-103

Abbreviations: allo-HSCT=allogeneic haematopoietic stem cell transplantation, GdE-= gadolinium enhancement negative, MFD= major functional disability, MSD=matched sibling donor, NFS=neurological function scale, NMSD= not a matched sibling donor, TPES= strictly 102 eligible transplant population, VLCFA=very long-chain fatty acids
Notes:
* Stable NFS is defined as NFS <=4 without an increase of >3 points from Baseline
¤Stable Loes is defined as a Loes score <=9 or not increasing a Loes score by >=6 points from Baseline. Rates of GHVD based on 'evaluable' patients.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The sought indication is: '*Skysona is indicated for the treatment of early cerebral adrenoleukodystrophy in patients less than 18 years of age, with an ABCD1 genetic mutation, and for whom a human leukocyte antigen (HLA)-matched sibling haematopoietic stem cell (HSC) donor is not available (see section 5.1)*.'

The target population is paediatric patients with CALD. In the clinical studies, males 4-14 years have been treated with Skysona. In addition, a few female cases have been described in the literature, and these are believed to have the same disease characteristics as the males, and thus the indication should include both sexes. The typical age for onset of MRI signs of demyelinisation and development of cerebral symptoms is 2-12 years, but there are rare cases with onset in children as young as 1 year and also in adolescents with the same disease characteristics as in children. Thus, patients younger than 3 years of age have not yet been treated with eli-cel; however, it is clear from literature on allogeneic and autologous transplantation that patients can effectively and safely undergo the required apheresis, conditioning, and infusion from the age of 6 months. In addition, data from 3 subjects ≤3 years of age in Study ALD-103 revealed prompt neutrophil engraftment with MFD-free survival at Month 24. No lower age cut-off for the Indication is needed since the clinical diagnosis of CALD including MRI detection of demyelination and neurologic function tests is age-limiting in itself. No child with a diagnosis of early CALD and without an available matched sibling donor should be excluded from treatment. As newborn screening becomes more available and earlier diagnosis of cerebral manifestations is possible, these patients will benefit from neurologic disease stabilisation at the earliest signs of brain damage. For the upper age cut-off, it is considered that adolescents have the same disease characteristics as children, whereas the rare adult patients with CALD have somewhat different disease characteristics, often with psychiatric problems, and CALD in adults may be superimposed on adrenomyeloneuropathy. Adults have not been included in the studied population. Thus, it is considered that the upper age cut-off of less than 18 years is appropriate. In summary, it is considered that efficacy may be extrapolated from the studied population to the intended target population.

Efficacy

MFD-free survival, a direct clinical observation of neurologic function, shows a compelling effect of eli-cel, with clinically meaningful results. NFS and neuropsychological testing, additional direct clinical observations of neurologic functions, show that most patients have minimal to no functional or cognitive decline, consistent with stabilisation of neurologic disease. MRI imaging biomarkers (Loes scores) reinforce these clinical observations.

A variability of efficacy has been noted in the comparison of subgroups with higher or lower Loes score than 4.5 at baseline. The few (n=4) subjects with a baseline Loes score ≥ 4.5 had a greater worsening of Loes score (+7.5, vs +1.0) and Performance IQ (-23 vs -2.5) from baseline to month 24 than subjects with a baseline Loes score of <4.5 (n=28). A sentence is included in the SmPC 5.1 to inform

the physician that patients with a higher MRI disease burden in terms of demyelination (and measured as Loes scores) tend to have a less favourable outcome. However, it is considered that the benefit-risk balance is positive for the entire studied patient population.

The primary endpoint MFD-free survival is considered a clinically relevant endpoint to capture survival without development of major disabilities arising from the rapid progression of cerebral demyelination. Given the poor prognosis in untreated CALD, eli-cel treatment is clearly superior to no treatment. In addition, the Month 24-MFD-free survival after eli-cel appears slightly superior than after allo-HSCT without a matched sibling donor (i.e. the NMSD subgroup of study ALD-103) and similar to that of allo-HSCT with a matched sibling donor (i.e. the MSD subgroup of study ALD-103).

After allo-HSCT, even when the donor is a matched sibling donor, there is a risk of engraftment failure and acute or chronic GvHD, which led to a decrease in the month 36 overall survival for the allo-HSCT MSD subgroup due to immunity-related death (chronic GvHD in at least one subject). Accordingly, eli-cel seems to offer a clinically meaningful advantage over allo-HSCT. For the subgroup of patients who do not have an available matched sibling donor (NMSD), i.e. the intended target population for Skysona, eli-cel offers a clear advantage in terms of MFD-free survival up to month 48 after treatment with an MFD-free survival of 90% for eli-cel and 59% for the allo-HSCT subgroup NMSD.

In addition to MFD-free survival data, neuropsychological data show a stabilisation of performance IQ for the majority of treated subjects, which shows that cognitive functions are preserved. The neuropsychological test results add to the whole picture of disease stabilisation after treatment for the majority of the patients. Clinical benefits are supported by pharmacodynamic endpoints showing the presence of integrated vector sequences (vector copy number, VCN) and the expression of the transgene (%ALDP+ cells), showing persistence of transduced haematopoietic stem cells over many years. Disease stabilisation is not immediate, but rather happens approximately one to two years after treatment, consistent with observations from allo-HSCT.

In summary, results for the primary endpoint MFD-free survival at month 24 have been provided for 30 Skysona-treated patients from the pivotal study ALD-102, exceeding the pre-specified benchmark for untreated patients and in line with the results from studies ALD-101 and ALD-103 on allo-HSCT treated patients with a matched sibling donor. In addition, performance IQ data show that cognitive functions are preserved in the majority of treated patients.

The effect is durable for up to 5-6 years, as shown by sustained MFD-free survival, stable NFS and cognition over time over follow up. At the latest data cut-off, 51 subjects have been followed for a median (min, max) 29.11 (0.1, 82.7) months. In the pivotal study ALD-102 followed by the long-term follow up study LTF-304, the median (min, max) duration of follow-up was 38.59 (13.4, 82.7) months. Nearly half of subjects (20/51) have been followed for at least 3 years, with 11 subjects followed for at least 5 years.

It is concluded that the efficacy results are considered sufficiently characterised for a full approval of the sought indication, which excludes patients with an available matched sibling donor (MSD).

Safety

Similar to allo-HSCT, treatment with eli-cel is preceded by significant procedural and medical interventions, including haematopoietic stem cell collection and myeloablative conditioning. Thus, in general, these risks of eli-cel are expected to be in line with the safety profile known to be associated with these procedures, and be reflected in the SmPC, including a description of which conditioning regimen was used in the pivotal study ALD-102. However, given that eli-cel consists of autologous transduced cells, this treatment compares favourably over allo-HSCT for subjects without a matched sibling donor.

Currently, data on the clinical effects only exist for patients treated in study ALD-102. In study ALD-104, which started later, a different conditioning regimen of busulfan and fludarabine is used, and there have been two cases of pancytopenia and a possible tendency for later platelet engraftment in this study. Apart from this, the safety profile between the two conditioning regimens appear similar and the final results of this study will be submitted in accordance to the RMP.

Eli-cel treated patients, as opposed to allo-HSCT treated patients, did not suffer from Graft-versus-host-disease (GvHD) after treatment as compared to 26/59 (44.1%) subjects who received allo-HSCT in TP-103. Based on the entire TP population, 49 of 51 eli-cel treated subjects (96.1%) achieved neutrophil engraftment compared to 53 of 59 (89.8%) of allo-HSCT patients. No subjects treated with eli-cel experienced either primary or secondary neutrophil engraftment failure (compared to 10/59 (16.9%) subjects treated with allo-HSCT). Most subjects treated with eli-cel, 47/51 (92.2%) subjects, achieved platelet engraftment at the time of the data cut-off date, compared to 47/59 (79.7%) subjects treated with allo-HSCT.

One death (1/45, 2.2%) was reported in TP-102/104 and a second subject died after receiving allo-HSCT off study. None of these deaths was considered related to eli-cel. In comparison, 15/59 (25.4%) subjects died in TP-103 (12 after first allo-HSCT and 3 after second allo-HSCT), 9 of whom had transplant-related mortality (TRM). Thirteen of the 15 deaths were reported in subjects without a matching sibling donor.

Thus, the current safety data seem favourable in the context of the intended population.

After autologous HSCT with lentiviral vector-based gene transfer, there are long-term uncertainties to be addressed relating to efficacy and safety, e.g. potential loss of efficacy due to loss of the transgene and insertional oncogenesis.

Formation of dominant clones has been detected in two subjects and the enrichment in the overall population in MECOM insertions has raised concerns with regards to the risk of insertional oncogenesis and the overall benefit-risk balance of Skysona. Transcription analysis of the affected subjects reveals dysregulation of the *MECOM* gene, resulting in increased levels of a subset of *MECOM* transcripts, the mechanism of which and clinical relevance is currently not clear. Recently provided (31 March 2021) updates one subject confirmed persistence of this predominant clone at the Year 6 evaluation. Both subjects continue to be clinically stable with no evidence of malignancy.

While the applicant is suggesting that is unlikely that the MNDU3 promoter is directly controlling MECOM expression, this is the most plausible explanation in line what has previously been reported for X-CGD and Wiskott Aldrich clinical trials. The position of the integration of the two dominant clones in two subjects is in the same region of MECOM (intron 2) of dominant IS in patients with X-CGD and Wiskott-Aldrich Syndrome that eventually developed AML after 2-3 years from the initial clonal dominance. Internal viral promoters have been described in preclinical models to influence expression of nearby genes even when placed internally in self-inactivating vectors. On the other hand, X-CGD and Wiskott-Aldrich Syndrome may carry additional intrinsic risk factors as compared to X-ALD cells. Thus, it is possible that these dominant clones will remain benign, such as in most cases of clonal haematopoiesis in aging, without evolving in leukaemia.

The potential risk of insertional oncogenesis remains, however, and will need to be handled in the RMP.

Current risk mitigations included in the SmPC 4.4 include at least annual monitoring for myelodysplasia, lymphoma or leukaemia with full blood counts. This appears to be appropriate.

In addition, ISA monitoring will continue in the ongoing and planned clinical studies. The current data are not sufficient for evaluating the long-term safety including any potential malignancies related to the therapy. Accordingly, there is a need for comprehensive post-approval safety data.

The ongoing long-term follow-up study LTF-304 together with the planned post-authorisation PAES-study REG-502 will follow eli-cel treated subjects for up to 15 years post treatment.

The applicant's plan to generate additional real-world clinical evidence in a post-approval setting, utilising the EBMT registry as the main data source for Study REG-502 has been previously discussed (most recently EMA/CHMP/SAWP/140510/2020). There are currently no established and active national disease CALD/ALD registries in existence that would fulfil the regulatory expectations for post-marketing commitments. In view of the current state of development of the registry study design and lack of alternative existing disease-based registries, this is acceptable.

However, it would be important to ensure that all necessary documents and procedures will be established in a timely manner so that these requirements will be met and allow the study conduct to get all the information required without delay.

In general, the proposed Pharmacovigilance activities appear to be appropriate and proportionate to the risks to be addressed.

Thus, the submitted data is considered appropriate to recommend a approval for the proposed indication, together with the obligation to perform two long-term follow-up studies LTF-304 and REG-502. In addition, the applicant committed to provide long term follow up data from the ongoing clinical studies ALD-102 and ALD-104.

3.7.2. Balance of benefits and risks

The benefit/risk balance is positive.

3.8. Conclusions

The overall Benefit/Risk ratio of Skysona 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 Skysona is favourable in the following indication:

Skysona is indicated for the treatment of early cerebral adrenoleukodystrophy in patients less than 18 years of age, with an *ABCD1* genetic mutation, and for whom a human leukocyte antigen (HLA)-matched sibling haematopoietic stem cell (HSC) donor is not available The CAT therefore recommends the granting of the marketing authorisation subject to the following 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 considers by consensus that the benefit- risk balance of Skysona in the above indication is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

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 regarding 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.

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 and contextualise the long-term safety and efficacy of Skysona in patients with cerebral adrenoleukodystrophy (CALD), the MAH should conduct, and submit the results of a prospective observational Registry Study (REG-502) of patients with CALD treated with Skysona or allogeneic haematopoietic stem cell transplantation (allo-HSCT) according to an agreed protocol (Stargazer).	Interim reports to be submitted in accordance with the RMP. Final report:2042
In order to evaluate the long-term efficacy and safety of Skysona in patients with cerebral adrenoleukodystrophy (CALD), the MAH should submit final results of Study LTF-304.	Interim reports to be submitted in accordance with the RMP. Final report:2037

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 elivaldogene autotemcel 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.

Paediatric Data

Furthermore, the CAT/CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0290/2018 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.