13 November 2025
EMA/CHMP/364628/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Waskyra

International non-proprietary name: etuvetidigene autotemcel

Procedure No. EMEA/H/C/006525/0000

Note

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

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List of abbreviations

AE	Adverse event
ANC	Absolute neutrophil count
AUC	Area under the curve
BM	Bone marrow
Bp	Base Pair
cDNA	Complementary DNA
CD	Cluster of differentiation
CD3i	Immobilized CD3
CFU	Colony forming units
CFU-C	Colony Forming Unit - Cells
CI	Confidence interval
CI UM	Control Group Designation: Unmanipulated
CI UT	Control Group Designation: Untransduced (Mock)
COI	chain of identity
cDNA	complementary DNA
COVID-19	Coronavirus disease 2019
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CUP	Compassionate Use Program
DP	Drug product
EAP	Expanded Access Program
EGFP	Enhanced Green Fluorescent Protein
ELISA	Enzyme-Linked Immunosorbent Assay
ELTFU	Extended long-term follow-up
EMA	European Medicines Agency
FACS	Fluorescent Activated Cell Sorting
G-CSF	Granulocyte-colony stimulating factor
GT	Gene therapy
GTMP	Gene Therapy Medicinal Product
GvHD	Graft versus host disease
gRV	Gamma Retrovirus /Retroviral
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HE	Hospital Exemption
HEK-293	human embryonic kidney 293 cell line
HERV :	human endogenous retroviruses
HIV	Human immunodeficiency virus
HIV-1	Human Immunodeficiency Virus Type 1
HLA	Human leukocyte antigen
HSC	Hematopoietic Stem Cells
HSCT	Hematopoietic stem cell transplantation
HSPC	Hematopoietic stem and progenitor cell
HTLV-1	Human T-lymphotropic virus 1
HTLV-2	Human T-lymphotropic virus 2

ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
Ig	Immunoglobulin
IgRT	Immunoglobulin replacement therapy
IL	Interleukin
IV	Intravenous
IVIG	Intravenous immunoglobulin
ITT	Intent-to-treat
KO	Knock-Out
LVV	Lentiviral vector
LTR	long terminal repeat
MMLV	Moloney Murine Leukemia Virus
MOI	Multiplicity of Infection
mAb	Monoclonal Antibody
mPB	Mobilized peripheral blood
mRNA	Messenger RNA
MPV	Mean platelet volume
NK	Natural killer
OLTFU	Observational long-term follow-up
OTL	Orchard Therapeutics Limited
p24	HIV p24 viral core structural protein
PB	Peripheral blood
PBSC	Peripheral blood stem cell
PCR	Polymerase chain reaction
qPCR	Quantitative Real Time Polymerase Chain Reaction
PedsQL	Pediatric Quality of Life Inventory
PPE	personal protective equipment
PYO	Person-year(s) of observation
Rag2	Recombination-Activating Gene 2
RCL	replication competent lentivirus
RIC	Reduced intensity conditioning
RNA	Ribonucleic Acid
RRE	Rev Response Element
SAE	Serious adverse event
SIN	self-inactivating
SMQ	Standardized Medical Dictionary for Regulatory Activities Query
SR-TIGET	San Raffaele Telethon Institute for Gene Therapy
TCR	T Cell Receptor
TI	Test Group Designation: Transduced
TPO	Thrombopoietin
TU	Transducing Units
UCB	Umbilical Cord Blood
UM	Unmanipulated
USA	United States of America
UT	Untransduced (or Mock)
VCN	Vector copy number

VSV	Vesicular Stomatitis Virus
VSVG	Vesicular Stomatitis Virus G-Protein
WAS	Wiskott-Aldrich syndrome
WASP	Wiskott-Aldrich syndrome protein
WAS	Wiskott-Aldrich Syndrome Gene
WHV	Woodchuck Hepatitis Virus
WIP	WASP Interacting Protein
WKO	WASP Knock-Out
WPRE	Woodchuck Hepatitis Virus Post-Transcription Regulatory Element
WPREmut6	Mutated Woodchuck Hepatitis Virus Post-Transcription Regulatory Element
WT (or wt)	Wild Type

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Fondazione Telethon Ets submitted on 24 November 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Waskyra, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 22 February 2024.

Waskyra, was designated as an orphan medicinal product EU/3/12/998 on 6 June 2021 in the following condition: Treatment of Wiskott-Aldrich syndrome.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Waskyra 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:
Waskyra | European Medicines Agency (EMA)

The applicant initially applied for the following indication: Treatment of Wiskott-Aldrich Syndrome in patients aged 6 months and older who have a mutation in the WAS gene and for whom no suitable HLA-matched related HSCT donor is available. This indication was further amended during the assessment as follows:

Waskyra is indicated for the treatment of patients aged 6 months and older with Wiskott-Aldrich Syndrome (WAS) who have a mutation in the WAS gene for whom haematopoietic stem cell (HSC) transplantation is appropriate and no suitable human leukocyte antigen (HLA)-matched related haematopoietic stem cell donor is available.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that genetically modified autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced *ex vivo* using a lentiviral vector encoding the human Wiskott-Aldrich Syndrome (WAS) gene was considered to be a new active substance.

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).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0083/2016 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0083/2016 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. 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.

1.4.2. New active Substance status

The applicant requested the active substance genetically modified autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced *ex vivo* using a lentiviral vector encoding the human Wiskott-Aldrich Syndrome (WAS) gene 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.

1.5. Protocol assistance

The applicant received protocol assistance as follows:

SA procedure	Areas	SAWP-Coordinators	Content
EMEA/H/SA/2996/1/2014/ADT/III	Non-clinical, Clinical	Jens Reinhardt	The protocol assistance addressed the following issues: -Sufficiency of the non-clinical programme - Acceptabilty of the single-arm study design, the choice of co-primary endpoints, study size, cell sourcing,
EMEA/H/SA/2996/2/2020/PA/SME/ADT/III)	Quality, Non-clinical, Clinical	Sheila Killalea, Jens Reinhardt	The protocol assistance addressed the following issues: - Acceptabilty of the phase 3 single arm open label study, duration of the follow-up.

1.6. Steps taken for the assessment of the product

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

CAT Rapporteur: Violaine Closson Carella CAT Co-Rapporteur: Joseph DeCourcey

CHMP Coordinator (Rapporteur): Jean Michel Race CHMP Coordinator (Co-Rapporteur): Jayne Crowe

The Rapporteur appointed by the PRAC was PRAC Rapporteur: Jo Robays

The application was received by the EMA on	24 November 2024
The procedure started on	24 December 2024
As the ATMP is a combined ATMP, the CAT agreed to consult the national Notified Bodies on the Environmental Risk Assessment of the GMO as the ATMP is a gene therapy product. The consultation procedure closed on	16 April 2025
The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	17 March 2025
The CAT Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	31 March 2025
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	31 March 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT during the meeting on	10 April 2025
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	16 April 2025
The applicant submitted the responses to the CAT consolidated List of Questions on	14 July 2025
The CAT Rapporteur circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	19 August 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	04 September 2025
The CAT agreed on a list of outstanding issues in writing to be sent to the applicant on	12 September 2025
The applicant submitted the responses to the CAT List of Outstanding Issues on	08 October 2025
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	23 October 2025
The outstanding issues were addressed by the applicant during an oral explanation before the CAT during the meeting on	05 November 2025
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 Waskyra on	07 November 2025
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 Waskyra on	13 November 2025

Furthermore, the CAT and CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	13 November 2025

2. Scientific discussion

Wiskott-Aldrich Syndrome (WAS) is caused by mutations in the WAS gene, which encodes WASP. This protein is a cytoskeletal regulator expressed specifically in the cytoplasm of hematopoietic cells including thrombocytes and leukocytes; it is essential for multiple cellular functions including adhesion, migration, phagocytosis, immune synapse formation, and receptor-mediated cellular activation processes.

A functional deficit of WASP is often associated with reduced number, size, and function of platelets, reduced function of T lymphocytes, impaired antibody production (especially to polysaccharide antigens), defective NK cell function, and reduced chemotaxis of phagocytes and dendritic cells.

2.1. Problem statement

2.1.1. Disease or condition

Wiskott-Aldrich Syndrome (WAS) is an X-linked primary immune deficiency and platelet disorder, characterised by thrombocytopenia and associated bleeding, eczema, recurrent infections, with increased susceptibility to autoimmunity, and lymphoreticular malignancies (lymphomas, leukemias, and myelodysplasias).

Wiskott-Aldrich Syndrome is a life-threatening illness associated with a severely reduced life expectancy and median survival of 14.5 years without definitive intervention; the majority of patients fail to reach adulthood.

2.1.2. Epidemiology

WAS is a rare disease, with an overall incidence estimated between 1/100,000- 1/1,000,000 live male births.

2.1.3. Biologic features Aetiology and pathogenesis

WAS is due to hemizygous mutations in the WAS gene (Xp11.4-p11.21), coding for the Wiskott-Aldrich Syndrome protein, exclusively expressed in hematopoietic cells and having a major role in the reorganization of the actin cytoskeleton, signal transduction and apoptosis.

Mutations in WAS can manifest with variable severity ranging from mild phenotype (XLT, score 1–2) to a severe phenotype (WAS, score 3–5), characterised by cellular and humoral defects and an increased risk of developing autoimmunity and malignancies. Over 400 unique mutations have been reported in the WAS gene, with a strong and complex genotype/phenotype correlation between WAS genetic variation and disease score/severity.

2.1.4. Clinical presentation, diagnosis

The 3 main clinical features of WAS are:

- Bleeding episodes, due to morphological abnormalities and reduction in the number of platelets as a consequence of WAS mutation. Bleeding episodes affect all patients with WAS (with mild or severe phenotype) and 30% of patients present with severe bleeding episodes.
- Infections: patients with WAS have a high susceptibility to infection.
- Eczema affects 80% of patients with WAS and the typical skin lesions resemble acute or chronic eczema in appearance and distribution.

The severity of the disease has been historically assessed using the Zhu-score system, which is based on the presence of thrombocytopenia, eczema, immunodeficiency, infections, autoimmunity, and/or malignancies.

Wiskott-Aldrich Syndrome represents a continuum of dysfunction from mild to severe disease due to various degrees of WASP deficiency/loss of function; the underlying disease pathophysiology is common to all phenotypic forms of WAS. Clinically, patients with severe WAS typically present with petechiae, bloody diarrhea, severe eczema, and recurrent, often life-threatening, infections.

2.1.5. Management

Current treatment options consist of conventional symptomatic and preventive management and haematological stem cell transplant (HSCT), which can be disease-stabilising when successful. Conventional supportive treatments, which only manage the clinical manifestations of the disease, include bleeding prophylaxis with platelet transfusions, antifibrinolytic agents or off-label thrombopoietin (TPO) receptor agonists, antimicrobials (for infection prophylaxis or treatment), immunoglobulin replacement therapy (IgRT), and immunosuppressive drugs, corticosteroids, and anti-CD20 monoclonal antibody rituximab for the management of autoimmune diseases. The TPO agonists romiplostim and eltrombopag were approved by EMA in 2009 and 2010, respectively, for the treatment of adult immune thrombocytopenic purpura and have been used *off-label* for the treatment of thrombocytopenia in patients with WAS. Splenectomy can be an effective treatment for thrombocytopenia, but it carries a significant long-term risk of bacterial sepsis and may not be effective in the setting of autoimmune thrombocytopenia.

Allogeneic HSCT, in which stem cells are obtained from a matched or mismatched, related, or unrelated donor, is considered the only potential disease-modifying treatment for WAS. When successful, HSCT leads to an improvement in platelet count and immune function and to the resolution of eczema. However, HSCT is often associated with severe short- and long-term complications. Common complications post-transplant include conditioning-related toxicity, graft failure and graft versus host disease (GvHD), with approximately 35% of patients developing acute GvHD and approximately 15% chronic GvHD, despite most patients receiving prophylactic immunosuppressive treatment. Graft failure is a severe complication and may be life threatening, requiring a second HSCT procedure in approximately 5% of patients. Autoimmunity is also reported in approximately 15% transplanted patients after HSCT.

HSCT with grafts other than from matched related donors (MRDs) carries the significant risks of worse survival, graft failure, and transplant-related morbidities, including GvHD. Only about 30% of patients have access to an MRD, and availability of a 10/10 matched unrelated donor is largely dependent on race and haplotype. In addition, outcomes for patients >5 years of age are still less favourable than those of younger patients, placing an urgency on early treatment.

The absence of suitable donors, the significant risks associated with transplantation, and the requirement for post-transplant immunosuppression therapy to prevent GvHD indicate an unmet medical need for novel therapies with transformative potential for subjects with WAS. The EBMT/European Society for

Immunodeficiencies guidelines advise there is a need for further approved treatment options for patients with WAS and gene-based therapies are promising approaches that have the potential to provide a functional cure in patients with WAS.

2.1.6. Feedback from patients' organizations

EMA engaged with patients' organisation in parallel to the assessment for feedback on any aspects that are of particular importance to patients/carers, such as quality of life, standard treatments and how acceptable they are, therapeutic/unmet medical needs, what benefits they would hope for in new medicines as well as what level of side effects they would consider acceptable.

The received feedback indicated the gene therapy to be an essential solution for children with no matched related donor.

2.2. About the product

Waskyra (etuvetidigene autotemcel) also referred as Telethon003, OTL-103, GSK2696275 is an Advanced Therapy Medicinal Product (ATMP) and was classified as a gene therapy medicinal product containing genetically modified cells. The active substance consists of a genetically modified autologous CD34+ cell-enriched population that contains HSPCs transduced *ex vivo* using a lentiviral vector (LVV) encoding the human WAS gene, which is intended for the treatment of WAS.

The mode of action of hematopoietic stem cell-based GT is the same as HSCT, which is to infuse into the patient HSPCs expressing WASP to correct the haematological and immunological defects present in patients with WAS. When infused into the patient following the administration of a reduced intensity conditioning regimen, the genetically corrected cells engraft and repopulate the hematopoietic compartment, giving rise to biologically active lymphoid and myeloid lineages expressing functional Wiskott-Aldrich Syndrome protein (WASP). Gene therapy, as an autologous procedure, does not require any donor search, is not associated with a risk of GvHD, does not require related preventive or curative measures, and can be performed after a reduced conditioning regimen (RIC), as incomplete chimerism does not pose any safety risk, thereby limiting the risk of conditioning-related toxicity.

It is intended for autologous use and should only be administered as a single dose via intravenous infusion. The minimum recommended dose of Waskyra is 7×10^6 CD34+ cells/kg. In clinical studies, doses up to 31×10^6 CD34+ cells/kg have been administered.

Waskyra must be administered in a qualified treatment centre by a physician with experience in haematopoietic stem cell transplantation (HSCT) and trained for administration and management of patients treated with the medicinal product.

2.3. Quality aspects

2.3.1. Introduction

The finished product (FP) is presented as dispersion for infusion containing 2–10 x 10^6 cells /mL of viable CD34+ enriched cell population as active substance.

Other ingredients are: dimethylsulfoxide, sodium chloride and human albumin solution.

The product is available in 50 mL ethylene vinyl acetate (EVA) infusion bag(s) with two available spike ports, packed in an EVA overwrap bag placed inside a metal cassette.

2.3.2. Active Substance

The section on the active substance is separated into two parts; part 1 for the lentiviral vector (starting materials) and part 2 for the transduced cells (active substance).

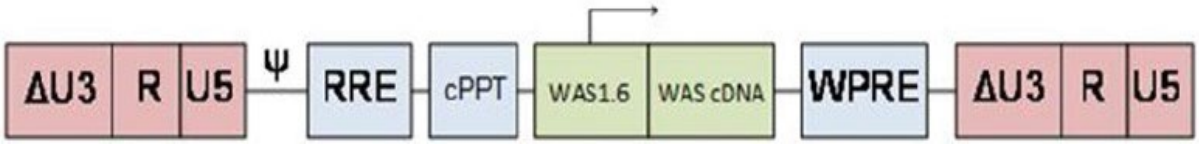
2.3.2.1. Part 1: Lentiviral (LVV) starting material

2.1.2.1.1 General information

The lentiviral vector encoding for the Wiskott-Aldrich Syndrome WAS protein (shortened WAS LVV) used for genetic modification of CD34+ patient cells is a third generation self-inactivating HIV1-based lentiviral vector pseudotyped with VSV-G, coding for the WAS protein under the control of the WAS promoter. It has been designed to minimise the risk of insertional mutagenesis and replicative particles. Its structure is adequately described and the WAS cDNA sequence is provided. The information provided by the applicant is considered adequate.

The construct is similar to a LVV used as starting material for another authorised product, with the same non transgene specific elements.

Figure 1. Schematic Representation of the WAS LVV



The LV is a critical starting material used to transduce the autologous cells to manufacture Waskyra.

Manufacture, process controls and characterisation [LVV]

Description of manufacturing process and process controls [LVV]

The lentiviral vector WAS LVV is manufactured at AGC Biologics S.p.A. in Bresso (Italy), which is also the manufacturer of genetically modified autologous CD34+ cells active substance (AS) and finished product. The manufacturing process B2 intended for commercial manufacturing is described with details of each step, including a flow-chart and description of the critical process parameters (CPPs), In Process Controls (IPCs) and In Process Specifications (IPS) and associated acceptable ranges, action limits and acceptance criteria. Definitions of the terms, CPP, IPC and IPS are provided. It starts with thawing of vials of HEK 293T WCB (Working Cell Bank) followed by several cell expansion steps before transfection with four constructs: the transfer vector plasmid pWASP coding for the WAS protein, and three packaging plasmids. Following transfection, viral particles are harvested and clarified. Clarified harvest is treated with benzonase and purified by anion exchange chromatography (ultrafiltration, and size exclusion chromatography. The SEC eluate is diluted with medium, sterilised by filtration, manually filled into cryovials and stored at ≤-65º C.

The listed parameters and target values are generally acceptable, and the justification for the proposed process parameter acceptance ranges are discussed further in the dossier.

The batch numbering system has been adequately described.

No reprocessing is described and no shipment of the LVV occurs as manufacture of the FP is performed at the same site.

Apparent discrepancies between CPPs for the manufacturing process (corresponding to PAR (Proven acceptable Range)) and for Continuous Process Verification (CPV, corresponding to NOR (Normal Operating Range)) have been explained and the manufacturing process is described with sufficient detail.

The action limit of the bioburden before sterile filtration appeared too high with regard to EMA guideline EMA/CHMP/CVMP/QWP/850374/2015 and to batch data. It has been tightened in accordance with the data presented in the dossier. Limitations for sampling are acknowledged.

Control of materials [LVV]

Four plasmids are used for transfection and the HEK293T cell banks used as cell substrate.

Raw materials are listed and in-housing testing on non-pharmacopeial materials is presented with acceptance criteria. The provided information is sufficient, the qualitative composition of complete medium IMDM and SCGM has been included and it has been clarified that $MgCl_2$ solution and Tris-EDTA are filtered 0.22 µm during manufacturing production to avoid contamination.

The details of the filters used throughout the LVV manufacturing process have been registered to in the updated version of the dossier.

Plasmids

Descriptions and detailed maps of the packaging plasmids and of the transfer plasmid pWASP are provided and the cloning strategy for the transfer plasmid is detailed. The manufacturing process is presented with sufficient detail. Plasmid specifications are in line with general monograph Ph. Eur. 3186 and general chapter Ph. Eur. 5.34. Examples of CoA (Certificates of Analysis) are provided for plasmids from each manufacturing site and comparability is sufficiently discussed in the section on Manufacturing process development. *E. coli* MCBs (Master Cell Banks) and WCBs production and characterisation are adequately described and comply with Ph. Eur. 5.34. Acceptance criteria have been implemented for the viable cell count of MCBs and WCBs of packaging plasmids. The induction of phage during plasmid manufacturing, which could result in inconsistent production is considered medium risk and mitigation measures are considered adequate. Plasmid stability data have been included and support the proposed plasmid shelf-life of 96 months at $\leqslant$ -65°C. As regards End of Production cells, the applicant has committed to establish and test EOPCs (end-of-production cells) for new WCBs. The WCBs currently in use to manufacture pWASP and packaging plasmids will be tested in 2026 and at the end of the upstream process as ongoing EOPC testing and banking, if a plasmid is manufactured within 5 years, which can be accepted. The proposed testing is acceptable, and acceptance criteria are presented.

HEK293T cell line

The cell substrate for LVV production is the HEK293T cell line. Its origin and history are sufficiently described. Three HEK293T master cell banks and three working cell banks have been produced for the manufacture of WAS LVV. Production and testing of the different MCBs and WCBs is described with sufficient detail. Overall, the testing panel is in line with Ph. Eur. 5.2.3. Controls of critical steps and intermediates [LVV]

The control strategy includes Critical Process Parameters (CPPs), In-Process Controls (IPCs), and In-Process Specification (IPS). CQA are defined and process monitoring is performed under a continued process verification (CPV) protocol. Viability and cell count methods used for the upstream process are appropriately validated. The information provided is considered sufficient.

Process validation [LVV]

The process validation is divided into 3 distinct phases: process design (Stage 1), based on small scale development studies and clinical manufacturing, Process Performance Qualification (PPQ), with three successful consecutive PPQ batches manufactured (Stage 2), and Continued Process Verification (CPV, Stage 3). The microbial control strategy comprises validation of storage conditions of media and reagents, aseptic process validation with media fills covering worst-case conditions and rooms intended for aseptic filling, demonstration of container closure integrity, sterilising filter validation and process microbial hold times. It is well described and considered overall adequate. Additional details including the number of vials filled and the duration of filling in order to demonstrate that the media fills are representative of the WAS LVV filling process were requested and have been provided by the applicant.

Validated process hold times are based on the shortest hold durations of the 3 PPQ batches. A cumulative in-process hold time study for which each process step duration and intermediate hold time was extended to the upper limit of the normal operating range was also performed.

The PPQ study involved the manufacturing of 3 PPQ batches at commercial scale according to Process B2. Some of the rooms which were used for manufacturing of PPQ batches are not available for commercial manufacturing and a comparability with post-PPQ batches manufactured in rooms intended for commercial manufacturing was performed (see discussion in section on Manufacturing process development). In addition, it has been clarified that no changes have been introduced between the LVV batches manufactured for the PPQ and post-PPQ to date. Overall, the PPQ study design is appropriate, and most results met the operating ranges and acceptance criteria. However, the results of two quality attributes (QAs), residual HCPs (host cell proteins) post-clarification pre-benzonase and residual LTA (Large T Antigen) protein in final WAS LVV, were above the acceptance criteria for 2 out of 3 PPQ batches HCPs were below the LOD in final WAS LVV and a clearance of more than 3 logs was shown. In addition, HCPs are negative or < LLOQ in all process B2 batches, except one batch. In addition, residual LTA protein levels are higher than the acceptance criterion defined for PPQ batches in some of the process B2 batches No definitive root cause was identified but it is indicated that limited data were available. Batch data indicate that several micrograms of LTA protein could be present in the amount of WAS LVV needed for a dose of finished product. Considering those results, the limited data available and the safety risk assessment and CD34+ transduction study presented , the omission of LTA protein testing from the commercial release specification of WAS LVV was not endorsed. In addition, it is considered that the data provided for plasmid DNA and HCP isn't sufficient for routine control. The applicant has updated the LVV specifications to include host cell protein (HCP) and residual plasmids DNA and has adequately justified the proposed release specifications. The applicant commits to performing the method validation for residual HCP and residual plasmid DNA as a post-approval commitment which is acceptable (REC 3).

Process yields and recovery appear consistent and acceptable to ensure a robust process.

Continued process verification (CPV) is sufficiently described. Critical process parameters that have been excluded from trending in CPV are justified.

Manufacturing process development [LVV]

The manufacturing process development and history is described with a clear overview of the changes made to the vector manufacturing process. Five process versions were used during the clinical studies: Process A1, A2, A3, A4, and B1. Version B2 is intended for commercial manufacturing.

The main changes were introduced between A4 and B1, with a change in manufacturing site, an upscaling, introduction of a new WCB, a change in plasmid manufacturer and replacement of porcine trypsin by TrypLE Select. Changes between Processes B1 and B2 are more limited and consist in the following process improvements: introduction of a new MCB and WCB, addition of a new manufacturer for packaging plasmids and scale-up of the depth filter kit for clarification. A rationale and impact assessment are provided for each change, and vector product quality attributes and finished product quality attributes were compared for introduction of each process version. The changes are considered justified and are supported by process characterisation and development studies. Overall, the data support the absence of impact of the changes on WAS LVV CQAs. Data have been added on WAS transgene expression at the level of WAS LVV and at the level of the finished product. These results support comparability of WAS LVV manufactured by process B1 and process B2, even if potency testing (transgene expression by Wes) ratios appear lower for FP batches manufactured with WAS LVV from process B1 (which can be considered as an improvement).Comparability between vector manufactured at the commercial scale (Process B1 and B2) and vector manufactured at the research scale (Process A1 to A4) is assessed in two studies, one at the level of LVV (study 1) and the other at the level of the finished product (study 2). In study 1, process A1, process A2, process A3 and process A4 were compared to process B1 and process B2 batches (including PPQ batches). Overall, the data support the comparability between Process A and Process B for WAS LVV, except for residual LTA protein. Indeed, levels of residual LTA protein are higher than the acceptance criterion defined based on process A for some process B2 batches. A safety assessment and side-by-side transduction studies using WAS LVV batches with high and low residual LTA protein levels manufactured by both Process A and B were performed. Results showed residual LTA protein levels < LOD in all finished product lots and no cytokine-independent abnormal growth due to neoplastic transformation with higher levels of residual LTA protein. Even if these studies are reassuring, the data are currently too limited. The applicant has specified that the assay that was used to measure residual LTA protein is no longer available because some reagents have been discontinued. Another assay is under development but currently shows low accuracy and high variability. According to the Applicant, the safety impact of residual LTA protein is considered low because of the protein half-life, the dilution during FP process and once administered to patient. The new method will be included in WAS LVV additional characterization. It is acknowledged that residual LTA protein cannot be included in release specification at the present time due to the issues encountered with the assay. The applicant has committed to implement the new analytical method for LTA protein residual quantification, and to re-test available batches and new batches of WAS LVV (REC 4). Depending on the results, residual LTA protein will be included in release specification or as characterisation test. In study 2, mobilised Peripheral Blood -derived CD34+ cells from one donor were split into two arms and transduced with WAS LVV derived from Process A (A3/A4 process) and B (B1/B2 process) in parallel. Overall, the results and statistical analysis support the comparability of finished product manufactured using vector from process A or B.

The pre-PPQ CQA risk assessment classified all process-related impurities as CQAs. The applicant has provided the requested justification for the declassification of release testing of process-related impurities from CQAs to QAs during the PPQ runs.

Characterisation [LVV]

Overall, the applicant has provided a high-level description of characterisation of the LVV.

The structure of the viral vector is described and the full sequence of WAS LVV provirus is provided. The applicant has committed to sequence WAS LVV from LTR to LTR (Long Terminal Tepeat) and to provide the sequence data to the Agency, which is acceptable (REC 2). The WAS transgene comprises the entire human WAS coding sequence under the control of the WAS promoter. All genetic elements are identified and

discussed. Vector integrity was determined by Southern Blot during clinical development but will be replaced by sequencing for commercial phase. The expected band was observed for all batches. Similarly, assays for transgene presence, lentiviral proteins and sequencing confirmed the expected results. As regards WASP expression, the qualitative western blot used during clinical development and the quantitative method using Wes instrument intended for commercial phase were described but no data were presented, the correlation between the level of expression of WASP determined in the LVV and in the finished product, as well as the link with biological activity of the finished product, was not discussed and the replacement of a WAS patient cell line with a surrogate WAS KO cell line was not discussed. The applicant has explained that the WAS KO cell line was a good surrogate of hematopoietic cells because it has immature characteristics and was widely used as a model to study the biology of hematopoietic cells. No correlation was found between the results of WASP transgene expression in WAS LVV batches and in FP batches. The applicant has justified this lack of correlation by the autologous nature of patients' cells and by the use of the same WAS LVV batch for different patients. Considering the variability of Wes ratio in FP from different patients using the same WAS LVV batch, it is indeed understandable that no correlation was identified. The information provided is considered sufficient for the WASP expression assay. Aggregation has been studied by DLS and the results indicate for most batches a homogeneous population of particles of the expected size. The applicant has stated that other methods for the study of vector aggregation including SEC with UV, SV-AUC and TEM were not suitable for the detection of aggregates. The applicant's justification based on prior knowledge is acceptable. These data, together with the fact that WAS LVV is used as starting material to transduce cells that will be subsequently washed, and prior experience are considered sufficient to conclude that the risk associated to aggregates is very low. Product-related impurities are presented as non-infectious viral particles. The discussion of the lack of impact of potential non-infectious particles on patient safety is acknowledged. The different process-related impurities are discussed.

2.1.2.1.2 Specification [LVV]

The commercial specification proposed for WAS LVV is overall acceptable. These cover potency/identity (Infectious titer, Infectivity, Physical viral titer, Transgene Expression and Transgene Sequence), safety (Adventitious viruses, endotoxins, mycoplasma, sterility, replication competent lentivirus and bovine viruses), general characteristics (Osmolarity, pH and clarity) and Impurities (residual plasmid DNA and Host Cell Protein).The choice of the cell line for the infectious titer assay and the change introducing an automatic cell counter for the RCL assay are sufficiently justified.

A summary of the changes to the methods during development is provided. Several of the tests have minor changes, in most cases to improve the assay, and this is supported.

Several methods are performed in compliance with Ph. Eur. And the non-compendial methods have been appropriately validated. Validation reports have been provided for each method.

Commercial specification is based on release results from several WAS LVV batches, using tolerance intervals, and on compendial requirements. Overall, the acceptance criteria are sufficiently justified for a vector used as starting material.

Reference standards or materials [LVV]

A primary in house reference standard has been established from process B2 batch. A new primary reference standard will be prepared, qualified, and maintained according to the protocol provided. The new primary reference standard will replace the current primary reference standard once it has been qualified. This is considered acceptable.

Container closure system [LVV]

The container closure system used for storage of WAS LVV is a single use 5.0 mL cryogenic cryovial polypropylene copolymer (PPCO) closed with a High-Density Polyethylene (HDPE) cap. The container closure components are pre-sterilised by gamma irradiation. The materials are biocompatible in accordance with USP Class VI, with negligible risk of leachables.

2.1.2.1.3 Stability [LVV]

Stability data are available for several WAS LVV batches with up to 60 months of storage at < -65°C. Stability data for Intermediate Storage conditions for 3 months for WAS LVV batches are presented. Finally, stability data for accelerated storage conditions (5±3°C) was also submitted. No significant downward trend was observed for any of the measured attributes on all analysed batches.

Stability for all batches were performed with the same container closure system as for WAS LVV intended for commercial use.

The stability protocols are acceptable. Justification of the choice of attributes as stability-indicating or relevant forced degradation data were requested to support the stability-indicating nature of the assays and the information provided is acceptable. All results comply with the acceptance criteria, except a few outliers for two parameters, and no trend is observed. The proposed shelf-life for WAS LVV is 48 months when stored at < -65°C, based on 48-month results for the 3 PPQ batches.

A post-approval stability protocol was submitted and it is considered acceptable.

2.3.2.2. Part 2: Transduced autologous CD34+ enriched population (active substance)

2.3.2.2.1. General information

Waskyra (*INN* etuvetidigene autotemcel) - is an autologous gene therapy medicinal product comprised of *ex vivo* genetically modified cells. The active substance is a genetically modified autologous CD34+ enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced *ex vivo* using a lentiviral vector encoding the human Wiskott-Aldrich Syndrome (WAS) gene. The genetically modified cells will contain one or more copies of the functional human WAS gene enabling the expression of a functional WAS protein (WASP).

The information related to the structure and general properties provided by the applicant is considered adequate.

2.3.2.2.2. Manufacture, characterisation and process controls

Manufacturers

The AS manufacturing site (AGC Biologics S.p.A) is provided. Information is in alignment with the flowchart of manufacturing steps in Annex 5.8 and the manufacturing site specified in the Application form (eAF). AGC Biologics S.p.A is in charge of the manufacture and QC testing for the active substance and the finished product, and of the finished product release. The GMP proof of compliance for AGC Biologics S.P.A. - Via Meucci, 3 Zambon Scientific Park 20091 Bresso (MI) is provided. The site was inspected by the Italian Competent Authority on February 12th, 2022.

Description of manufacturing and process controls

The description of the active substance manufacturing process includes flow charts, brief narratives, and tabular overviews of the critical process parameters (CPPs) and in process controls (IPCs). Briefly, the process consists of CD34+ cell enrichment, pre-stimulation, transduction, and cell harvest, in maximum four days.

Patient cells are sourced from mobilized Peripheral Blood (mPB). Immuno-magnetic selection is performed (step 1). Pre-stimulation of the CD34+ enriched cells is performed with suitable cytokines in retronectin coated bags (step 2). The transduction is performed by adding LVV in two steps at the same MOI. After the first transduction (step 3) the cells are then washed and re-seeded. After the second transduction (step 4), the cells are centrifuged and resuspended in fresh medium (step 6). The AS can be held at 2 - 8°C for up to two hours before preparation of FP.

Several manufacturing process details were requested and were provided.

The process controls are described; the applicant has provided the risk assessment performed to determine which process parameters and quality attributes were deemed to be critical. The applicant has implemented an IPS for microbiological control at various steps of the manufacturing process. The description of the overall microbiological control strategy is considered adequate.

Finally, the applicant lists PARs for process parameters. Data to support the PARs from this study have been provided.

A sufficient description of the batch size and numbering system has been given as this is a continuous batch process. No reprocessing is performed.

Controls of materials

Mobilized peripheral blood (mPB) and the lentiviral vector WAS LVV are used as starting material in the manufacture of the active substance.

An overview of the raw materials used for AS manufacturing is provided, including information on the manufacturer and specifications. A mix of in-house testing and CoAs are used to determine if the raw materials meet in-house specifications. This approach is considered acceptable and representative CoAs have been provided.

Information on the compatibility of the materials used in production with the cells and the potential extractables and leachables is provided.

The information provided on the autologous cells is sufficient. Procurement of mobilized peripheral blood (mPB) by qualified centres and compliance with relevant directives is confirmed. Virus screening, mycoplasma testing and compliance with EU directives 2004/23/EC and 2006/17/EC is confirmed by verification of documentation prior to the start of manufacturing.

Control of critical steps and intermediates

In process controls for the AS manufacturing process are in place. Microbiological control is performed on the starting materials. As the manufacturing process is short and includes several wash steps, it is agreed that no further in process tests are in place for microbial control. The finished product is tested for mycoplasma, endotoxin, and sterility.

Process validation and/or evaluation

The AS is manufactured to FP as part of a batch process without interruption. Therefore, process validation studies are located in the FP section.

Manufacturing process development

In total 4 different AS/FP manufacturing processes are identified (A1-A4). Differences include the manufacturing site, the starting material BM (Bone Marrow) or mPB, the CD34+ enrichment procedure, the presence or absence of an additional cryopreservation step for the CD34+ enriched cells, the scale for the WAS LVV, the container closure system, and the final formulation (fresh or cryopreserved). Process A4 was used for pivotal clinical study OTL-103-4 and is the proposed commercial manufacturing process. The applicant has performed a comparability study of CD34+ cells derived either from BM or mPB representative of the process.

Several batches derived from mPB and BM were compared. The statistical analysis suggested that there is no marked difference in any of the quality attributes examined, except for the percentage of $CD34^+$ and the clonogenic capacity which are significantly higher for mPB-derived FPs when compared to the data obtained from BM batches. These differences with FP batches manufactured with BM as the cellular source material represent an improvement of the manufacturing process. As conclusion, both mPB- and BM-derived FPs are deemed acceptable.

Furthermore, the applicant has performed a retrospective comparison of BM vs mPB-derived clinical FPs. The difference in terms of CD34+ percentage was confirmed with this study. There were no significant differences in the VCN or transduction efficiency, respectively, between FP produced from BM or mPB, demonstrating that the starting material does not have an impact on the transduction process.

Overall, it can be concluded that the manufacturing process results in comparable FP irrespective of whether CD34+ cells are derived from BM or mPB as a cellular source material.

The impact of freezing the enriched CD34+ cells (derived from both mPB and BM) prior to manufacture, on FP quality attributes, was also evaluated. It was concluded that the differences observed between FP manufactured from either fresh or frozen CD34+ cells were not significant and that the data were within the known clinical patient ranges.

In order to have the option to combine multiple harvests, it was proposed that multiple harvests could be held refrigerated and then pooled prior to the start of the enrichment step. Therefore, the applicant has performed a study to assess the shelf-life of mPB at 2-8°C prior to the start of AS manufacture. The results showed that the shelf-life of mPB can be extended to 104 hours at 2-8°C without impacting the quality of the FP. For commercial manufacture, 104 hours is proposed as the maximum hold-time of the starting material.

A comparison of the manufacturing platforms was carried out. Differences between the outputs were deemed to be insignificant.

Characterisation

In this section, descriptions of the WASP transgene expression assay, immunophenotype, VCN, CFU, transduction efficiency, Integration site analysis, Endotoxins and Mycoplasma are provided. The results for these assays are provided.

The active substance has been sufficiently characterised by physicochemical and biological state-of-the-art methods.

2.3.2.2.3. Specification

Specification

All release testing of Waskyra is performed at the stage of Finished Product; therefore, a specification for Active Substance is not required.

Reference standards or materials

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

Container closure system

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

2.3.2.2.4. Stability

The active substance and finished product processes are continuous and therefore no information is provided on the AS. Please refer to the finished product stability section below.

2.3.3. Finished Medicinal Product

2.3.4. Description of the product and pharmaceutical development

Description of the product

The FP dispersion for Infusion is composed of 10 – 20 mL of cryoformulation medium (DMSO, HSA, and saline solution) containing 2-10 x 10^6 CD34+ cell transduced *ex vivo* using a lentiviral vector encoding for the human WAS cDNA sequence per ml. The product is presented cryopreserved in EVA bag(s). The number of EVA bags depends on the total amount of cells and will vary between individual patients. If required, up to eight 50 mL EVA bags may be used for one patient.

After thawing, the product is administered by intravenous infusion without further manipulation.

Control of excipients

The FP is formulated in DMSO, HSA in saline solution. DMSO and HSA comply with compendial monographs.

0.9% w/v Sodium Chloride Infusion is purchased as a medicinal product licensed by a European Union member state. The human serum albumin is registered as a medicinal product within the EU. The EU marketing authorisation number for these medicinal products used as excipients were provided upon request.

The applicant provides the in-house specifications for sodium chloride. Adequate justification of specifications is provided.

The only human origin excipient used in the manufacture of Waskyra dispersion for infusion is Human Serum Albumin (HSA). Two sources of HSA are used. A side-by-side comparison on the FP manufactured using the two different HSA was performed using a pooled t-test. FP formulated with HSA #1 is considered comparable to the FP formulated in HSA #2.

Formulation development

Initially Waskyra was formulated as a fresh finished product (i.e. not frozen). Subsequently, it has been formulated to produce a cryopreserved finished product. The development of a cryopreserved formulation is in principle endorsed. Comparability with the fresh formulation is discussed in the section below.

There is no overage.

Manufacturing process development

The finished product (FP) manufacturing process has evolved, from process A1 to process A4. Process version A4 is the intended validated commercial process for the cryopreserved mPB-derived FP. Implemented changes through the process development include a change to the FP formulation, container closure system and the associated FP filling process, a manufacturing site change, and the change of HSA source.

FP batches manufactured with the process version A4 were used for clinical study OTL-103-4 (10 patients treated).

CMC data supporting comparability involve three studies: a study with HD material comparing product derived from BM and mPB, a split study with HD material comparing fresh FP from previous processes and site versus cryopreserved FP from A4 process and site, and a comparability assessment of quality attributes on clinical batches. Data to support the use of healthy donor cells as a surrogate for patient cells are provided.

The overall approach to assess comparability is endorsed. Overall, the provided data appear to support comparability.

The applicant has provided a comparability study to account for batches manufactured following PPQ in a new room within the same site.

Container closure system

The primary packaging chosen for (FP) is an EVA bag, 50 mL nominal volume, with a recommended fill volume of 10 to 20 mL.

The choice of container closure system (CE-marked EVA bag) is considered adequate.

Microbiological attributes

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

Compatibility

An extractables/ leachables study from direct contact materials was performed.

The applicant states that a true leachables study cannot be performed as the final product contains patient specific cells. This can be agreed and the use of a simulated leachables study is acceptable.

2.3.5. Manufacture of the product and process controls

Manufacturers

Active substance and Finished Product are manufactured at the same site (continuous process).

Batch Formula

As the batch size and cell concentrations listed in batch formula have wide ranges, the applicant has added a table to the dossier demonstrating the relationship between FP cell concentration, FP volume and patient bodyweight.

Description of the manufacturing process and process controls

The finished product manufacturing process consists of cell wash, cell concentration, final formulation, filling, and cryopreservation. In general, sufficient detail on the procedures, critical process parameters and in process controls is provided. No hold times are defined in the finished product manufacturing process. No routine reprocessing procedure has been established at this time. The batch numbering system is described.

Traceability

Sufficient information is provided on traceability, including a flow diagram and brief descriptions of the different components (Chain of identity (COI) ID for each patient at finished product order, Donor identification number for cellular source material (DIN), shipment, receipt, manufacturing, storage, batch release). The FP label will contain the COI ID and batch number assigned to that specific COI ID.

Controls of critical steps and intermediates

The only IPC in the FP manufacturing process is a test for cell viability before addition of the cryopreservation medium. This is acceptable, as the FP manufacturing process is very short and only consists of cell wash, concentration, formulation, filling and cryopreservation. Microbiological control, endotoxin, and mycoplasma are tested at FP release, and results will be available prior to administration to the patient.

Process validation

Three consecutive PPQ batches were manufactured using HD material as per the commercial manufacturing process. The applicant provides a justification of the use of HD material in support of PPQ studies.

For the three PPQ batches, the parameters and attributes measured and tested throughout the production process met the set acceptance criteria or were within the expected range.

Media fills

No growth was observed in any of the samples from any of the media fills.

Impurity removal

The applicant evaluated removal of process related impurities during the wash steps after the transduction. The results of the evaluation for the three PPQ batches demonstrate that the wash steps are capable of reducing the levels of process related impurities.

The levels observed in the supernatants are generally considered to be safe for administration based on a safety assessment that was performed.

Container closure integrity

A container closure integrity test (CCIT) was performed to validate the integrity of the EVA bag used as the primary packaging.

A microbiological integrity test of the EVA bags was performed during stability.

Results of the CCIT testing have passed all acceptance criteria. At each time point, the test samples were sterile at the end of the incubation period, no growth was observed in the negative control, and the growth promotion sample showed contamination.

Transport validation

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

Continuous process verification (CPV)

A brief summary is provided of the continuous process verification (CPV) that will be implemented.

Nitrosamines

The applicant has assessed the risk of formation or introduction of nitrosamines in the manufacturing processes of FP and WAS LVV. The risk analysis demonstrates a low risk of the presence of nitrosamines in FP and WAS LVV. This conclusion is supported based on the provided risk evaluation as no risk has been identified with regard to the risk factors related to nitrosamine formation as outlined in the Questions and answers on CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products.

2.3.6. Product specification

Specification

The release specification for FP includes general tests (viable cell concentration, total volume, appearance), identity/purity by immunophenotype (% $CD34^+$), potency (viability, VCN per cell, VCN per transduced cell, transduction efficiency, clonogenic capacity), potency/identity (transgene expression by WASP transgene expression), and safety (sterility, bacterial endotoxins, mycoplasma).

Additional characterisation is performed as part of the continued process verification.

It was clarified that the specification presented is also applicable to stability studies.

The specifications outlined are largely aligned with Ph. Eur. 3186. Acceptance criteria and method identification numbers have been assigned.

The applicant has stated that particulate matter testing was evaluated for Waskyra and determined that the test is not technically suitable due to the type of product. Visual inspection is performed to assess visible particulate matter both during manufacturing and post-thaw at the clinical site where the infusion will be administered. The omission of particulate matter testing can be accepted.

The applicant has presented stability data demonstrating that FP stored in cryobags and vials in terms of QC results are comparable.

The difficulties with establishing a suitable potency assay are acknowledged.

The applicant is stating that different assay platforms have been evaluated to serve the purpose of finding a functional potency assay, but they all have failed to demonstrate WASP-dependent activity resulting in the unavailability of such a functional assay.

The WAS transgene expression method was improved in order to have a quantitative western blot.

The applicant has presented a correlation analysis of Waskyra attributes with primary and secondary efficacy parameters to assess if the potency panel associates with clinical outcomes in treated patients. As regards the capability of the matrix of assays to detect sub-potent batches, the applicant is arguing that all patients treated with Waskyra demonstrated satisfactory clinical efficacy, therefore no sub-potent batch was ever injected.

The applicant's rationale for omitting a functional potency assay from the proposed matrix assays is adequately supported.

Analytical Procedures, Validation of Analytical Procedures

An overview of the analytical methods is included. Viable cell concentration & viability, immunophenotype, clonogenic capacity as well as the safety relevant quality attributes endotoxin, sterility and mycoplasma are tested according to the respective Ph. Eur. monographs.

Validation reports for the methods used for the viability, the immunophenotype and the clonogenic capacity have been provided. These methods have been appropriately validated.

All other methods are in-house methods for which brief method descriptions have been included.

For the validation of non-compendial methods, adequate summaries of validations or validation reports were provided. Most of characteristics of analytical procedures (e.g. accuracy, precision, specificity, linearity, range, quantitation limit) were validated as per ICH Q2 requirements. These non-compendial methods have been appropriately validated.

Method suitability testing of microbiological control and non-compendial mycoplasma-NAT as well as generic validation of the non-compendial mycoplasma-NAT was not performed with the actual FP matrix. The applicant commits to validate the method with the FP matrix (Saline solution, HSA and DMSO) in a timely manner and to provide the obtained validation data (REC 6).

As already acknowledged by the applicant, the NAT-based mycoplasma testing is not validated and will be subjected to a planned change in the mycoplasma sampling point and a validation. The applicant proposed to address these deficiencies and changes with a post-marketing commitment (REC 7). The non-compendial analytical procedures are appearance, vector copy number and transgene expression.

As the appearance method is not currently performed in accordance with compendial requirements, the applicant has confirmed that method development will be conducted post-authorisation to align the method with USP and Ph. Eur. requirements. This is considered acceptable.

As regards transduction efficiency assay, the validation study does not include the range of 80-100%, which does not allow to have a numerical value for all batches. The applicant commits to develop and validate a new assay for the assessment of transduction efficiency allowing to obtain numerical values in the range of 80-100% of TE (REC 5).

Batch analysis

Information of AS/FP batches including process number, vector process number, starting material used (BM or mPB), manufacturing date and use of batch are provided.

The results in this section are presented alongside the current specification. All batches were released according to the specifications effective at the time of release; any differences are described in footnotes.

Characterisation of impurities

In this section, information is provided as regards product-related impurities (CD34- cells and non-viable cells) and process-related impurities (vector-process related impurities and cell-process related impurities). As viable cells and percentage purity of $CD34^+$ are controlled at release, these impurities can also be considered as indirectly controlled at release.

A justification is provided specifically for phenol red and anti-CD34 selection beads, which are not studied in PPQ batches.

The applicant has identified phenol red as an impurity with genotoxic potential. The calculated dose of phenol red present in Waskyra per dose exceeds the TTC of 1.5 µg/day. It also fails to meet the ICH M7 less than lifetime dose of 120 µg/day. The applicant states that EMEA/CHMP/QWP/251344/2006 allows for greater flexibility for life threatening conditions. The applicant has provided justification for the continued use of SCGM media containing phenol red. The applicant outlines that the current manufacturing process is a well-established, validated process with a proven track record of safety. The risk associated with phenol red is low as, following clearance, the amount of phenol red is well below the PDE. And finally, that the introduction of a phenol red free medium would constitute a major change to the manufacturing process that would make monitoring of metabolic activity of the product and potential contaminations more difficult as monitoring a simple colour change is straightforward. Overall, the Applicant has suitably justified the use of SCGM media containing phenol red and it is agreed that the risks associated with the change to a phenol free media outweighs the benefits.

Justification of specifications

The acceptance criteria presented have been established based on compendial requirements, relevant regulatory guidance and clinical experience with Waskyra. A statistical analysis was conducted for quantitative release attribute to generate tolerance intervals.

The applicant justified the use of a potency panel (which includes the WASP transgene expression quantitative method (Wes) which confirms (and quantifies) the presence of WASP, together with viability, vector copy number, vector copy number per transduced cells, transduction efficiency and clonogenic capacity and this has been found acceptable.

Furthermore, no data is presented as regards the distribution of VCN in the transduced cells and the transduction efficiency (percentage of transduced cells and VCN) in the different subpopulations of the CD34+ cells. The applicant commits to include a standard curve in the VN and TE assays to allow calculation of VCN and TE in specific subpopulations from Ct (cycle threshold) values and to provide theoretical calculation of VCN and TE in specific subpopulations from historical Ct values (REC 1).

Reference standard or materials

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

Container closure system

The FP is filled into one or more sterile, single use, pre labelled EVA bag(s). Each primary bag is then sealed and packaged into an overwrap EVA bag which is also sealed, the packaged FP is then cryopreserved. The frozen packaged configuration is placed in a metal cassette for storage in the vapor phase of liquid nitrogen.

The provided information on the container closure system includes an overview of the raw materials of the different components of the primary container closure system and the manufacturer's specifications of the cryobags. A CoA and CE-certificate are provided.

The CryoMACS freezing bags are provided pre-sterilised using irradiation by electron beam. The applicant states that the validation of the sterilisation process complies with ISO 11137with results in accordance with Ph. Eur. 5.1.1 and the Guideline on sterilisation of the medicinal product, active substance, excipient and primary container (EMA/CHMP/CVMP/QWP/850374/2015), and is considered acceptable. The endotoxin acceptance criteria of the primary container closure is deemed acceptable in respect of the minimum primary container closure fill volume.

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

2.3.6.1. Stability of the product

The claimed shelf-life is 6 months when stored at < -130°C. Once thawed, the finished product can be kept for maximum 45 minutes at room temperature (20 °C to 25 °C).

Up to 6 months stability data are provided for four batches, with HD material, formulated at a cell concentration of 2 x 10^6 cells/mL or 10 x 10^6 cells/mL. In-use stability data at the beginning and end of shelf-life are available for three batches all formulated at 10 x 10^6 cells/mL). All results at 60 months met the proposed release specifications. It can be accepted that the stability claims are based on data obtained with healthy donor cells, as comparability of healthy donor cells and patient derived cells is sufficiently demonstrated.

It can be accepted that the stability claims are based on data obtained with a BM derived batch, as FP comparability is sufficiently demonstrated whether CD34+ cells are derived from BM or mPB as a cellular source material.

Parameters tested in the stability studies include pH, osmolality, sterility viability, cell concentration, immunophenotype (% $CD34^+$), clonogenic capacity, transduction efficiency, VCN, and transgene presence/expression. The test panel is considered appropriate. The deviations from ICH guidelines with regard to the time-points tested is justified by the limitation in the number of cells isolated. Nevertheless, the majority of acceptance criteria for stability studies have been defined as "report results".

As regards the in-use shelf-life for batches formulated at 2x10^6/mL, since no decreasing trend was observed at T=45 min, the in-use shelf-life for batches formulated at 2 × 10^6 cells/mL is currently proposed to be limited to 45 minutes.

A stability study with a clinical batch manufactured with patient's material, manufactured under hospital exemption is ongoing. Up to 2 months stability data are provided.

No meaningful downward trend was observed.

No post-approval stability studies are currently planned. Additional stability studies may be performed in support of process changes, process validation, and comparability studies.

In summary, with the overall data submitted, the finished product can be kept is 6 months when stored at < -130°C and for maximum 45 minutes at room temperature once thawed.

2.3.6.2. Post approval change management protocol

Change of equipment (peristaltic pump model for sterile filtration) and starting material (HEK 293T Master Cell Bank and Working Cell Bank) for WAS LVV manufacturing process:

A PACMP is submitted to introduce a new HEK293T WCB for the manufacture of WAS LVV and a new peristaltic pump for sterile filtration due to discontinuation of the previous model.

For the change in peristaltic pump, the pump speed will be adapted to ensure the same flow rate. The applicant has clarified that introduction of the new peristaltic pump will not change the flow rate but the rpm speed.

Considering all the information provided the PACMP and the submission of a type IB variation post-approval can be accepted.

2.3.6.3. Adventitious agents

Non-viral adventitious agents

The proposed microbial control (non-viral adventitious agents) of the lentiviral vector, the autologous cells and the final finished product, including control of mycoplasma, other bacteria, and fungi is deemed acceptable and is sufficiently described.

The provided results (also presented in the CoA) are within the acceptance criteria.

Risk of contamination with animal TSE

Starting materials of the WAS LVV

The applicant was requested to provide further information regarding manufacturing process of the bacterial banks, plasmids, medium and its composition. Appropriate data was provided.

The proposed microbial testing (sterility, bioburden, mycoplasma) for the plasmids (MCB, WCB and plasmid batches) and the HEK293T cell line (MCB and WCB) used to manufacture the WAS LVV is acceptable.

The certificates of analysis of the MCBs, WCBs and plasmids batches of the transgene plasmid pWASP and the packaging plasmids are provided. The results of microbial tests (sterility, bioburden and mycoplasma) are compliant.

The certificates of analysis of the HEK293T MCBs, WCBs and the PPCB are provided. The results of microbial tests (sterility, bioburden and mycoplasma) are compliant.

CoA of a new WCB proposed to be used is provided and CoA of the MCB from which this WCB has been manufactured are provided as requested.

Raw materials for the HEK 293T cells banks

The TSE Certificate of Suitability manufacturing process, certificates of irradiation and certificate of origin of FBS batches were provided. The certificate of analysis of reagents used for cell detachement is provided.

Raw materials for the WAS LVV

Data regarding FBS used in the manufacture of the TLT03 WAS lentiviral vector was provided and deemed acceptable. Certificates of origin and analysis of endonuclease, SCGM (including insulin and HSA) are provided.

Raw materials and excipient for the WAS FP

Appropriate certificates were provided for the reagent used for CD34+ cell enrichment, cytokines, SCGM and HSA, along with correspondent TSE statements

Adventitious viruses

HEK293T Cell Banks and LVV

The virus screening of the donor is satisfactory. The methods used for each virus were specified and described as requested.

The laboratories in charge of these controls were identified.

WAS FP

The information provided for the licensed medicinal products used during manufacture of active substance and final formulation of finished is satisfactory.

The donors are tested for HIV1/HIV2, HBV, HCV and Parvovirus B19.

As there is no viral clearance within the manufacturing process, control of adventitious agents within the raw materials is essential. It was requested to provided appropriate data in compliance with EMA/CAT/GTWP/671639/2008 Rev. 1, "*viral safety as well as measures taken to minimise the risk of transmitting agents causing Transmissible Spongiform Encephalopathies (TSE) of any reagent or material of animal origin should be adopted. Recombinant proteins such as enzymes, antibodies, cytokines, growth or adhesion factors should be characterised and controlled, where appropriate and relevant, in accordance with the principles described in Ph. Eur. 5.2.12*." A viral risk assessment was performed on all raw materials and excipients in accordance with Ph. Eur. 5.2.12 and Ph. Eur. 5.1.7 to identify any associated viral risk. A summary of the risk assessment was provided. The data is deemed acceptable.

Viral clearance studies

The absence of viral clearance steps in the manufacturing process is justified.

2.3.6.4. GMO

Environmental risk associated with Waskyra is considered negligible. Refer to Paragraph 2.4.5.

2.3.7. Discussion on chemical, pharmaceutical and biological aspects

The provided Quality documentation is of reasonable quality, and no major objections were raised. The information on the manufacturing process and its control is in general sufficient. A valid GMP certificate has been provided.

The manufacturing process is considered continuous from the active substance to the finished product, therefore for several active substance sections, no information is presented but can be found in the finished product sections.

Several changes were made to the manufacturing process of the virus vector and the AS/FP.

Overall, it can be concluded that the manufacturing process results in comparable FP.

The WAS lentiviral vector (WAS LVV) is a (VSV-G) pseudotyped replication-incompetent third generation self-inactivating (SIN) HIV-1 - based lentiviral vector that has been modified to carry the WAS protein cDNA sequence for transduction of CD34+ cells. An adequate description of the vector manufacturing process is provided.

The WAS LVV process has been validated in 2020. However, due to changes in manufacturing rooms, at least one PPQ batch manufactured in the rooms intended for commercial manufacturing was requested to support process validation. One validation batch has been manufactured in the rooms intended for commercial manufacturing as requested. Results of CPPs, CQAs, QAs, PAs, microbial in-process testing, release CQAs and extended characterisation are presented. Overall, the results support the comparability of the validation batch with PPQ batches.

A high-level description of the analytical procedures is provided in the dossier. In general, the approach to assay validation is in accordance with the ICH Q2(R2) and the results met the proposed acceptance criteria. The information on the container closure is sufficient. Several concerns were raised as regards the LVV stability data and were resolved.

As regards process validation of the AS/FP, the validation status of the process in the suite that will be used for commercial production has been demonstrated, in response to a request for supplementary data.

Characterisation of the transduced cells includes batch analysis data and extended testing results for all clinical batches. The extended testing includes immunophenotype, clonogenic potential, impurities, and integration site analysis.

As regards the in-use stability data for batches formulated at $2x10^6$cells/mL, , the in-use shelf-life will be limited at 45 minutes.

In addition, seven recommendations are proposed that have been described in this report (see section below). This are deemed not to affect the benefit/risk of this product.

2.3.8. 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 have been presented to give reassurance on viral/TSE safety.

2.3.9. 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 recommends the following points for investigation:

Recommendations pertain to quality
1. For finished product, a standard curve should be included in the VN and TE assays to allow calculation of VCN and TE in specific subpopulations from Ct values. Theoretical calculation of VCN and TE in specific subpopulations from historical Ct values should be provided.

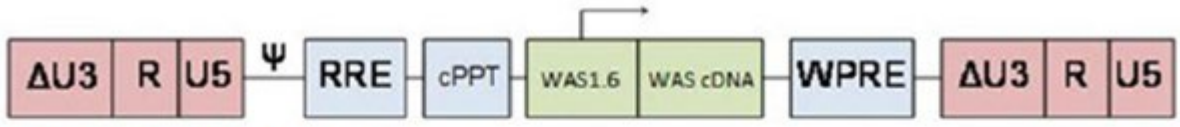
Recommendations pertain to quality
2. The sequence data of WAS LVV from LTR to LTR should be provided.
3. The applicant should commit to submitting method validation for the residual HCP and residual plasmid DNA assays for release of the LVV.
4. A variation should be submitted with qualification data and batch results, to support residual LTA protein assay as a characterisation or release test.
5. The applicant should commit to develop and validate the new transduction efficiency assay including the range of 80-100% in a timely manner and a variation should be submitted to support this new assay.
6. The applicant should commit to validate the microbiological release testing method with the Waskyra finished product matrix (Saline solution, HSA and DMSO) in a timely manner and a variation should be submitted to provide the obtained validation data.
7. The applicant should commit to include the pre-enrichment step in the validation of the NAT-based mycoplasma testing method. For this, the pre-enrichment step could be included in the preparation of the spiked cells with spiked Mycoplasma and the extraction of the DNA by the sub-contractor laboratory, Eurofins, before to send the DNA to AGC Biologics A variation should be submitted to provide the obtained validation data.

2.4. Non-clinical aspects

2.4.1. Introduction

Telethon003 is a gene therapy medicinal product (GTMP) comprised of a genetically modified autologous CD34+ cell enriched population containing hematopoietic stem and progenitor cells (HSPCs) transduced ex vivo using a lentiviral vector (LVV) encoding the human Wiskott Aldrich Syndrome (WAS) cDNA sequence under the control of an endogenous WAS gene promoter (referred to as WAS LVV).

Figure 2 Schematic representation of the WAS LVV provirus in transduced cells



ΔU3 = HIV-1 LTR unique in 3' region
R = HIV-1 LTR region
U5 = HIV-1 LTR unique in 5' region
Ψ = HIV-1 extended encapsidation signal
RRE = HIV-1 Rev response element
cPPT = HIV-1 central polypurine tract
WAS1.6 = 1.6kb sequence of endogenous human *WAS* promoter
WAS cDNA = Human Wiskott-Aldrich Syndrome gene cDNA
WPRE = Woodchuck hepatitis virus posttranscriptional regulatory element (mut6 variant)

The rationale for gene therapy is that autologous, gene corrected CD34+ HSPCs can stably engraft, self-renew and differentiate appropriately into multiple hematopoietic lineages. Gene-corrected cells producing

intracellular WASP demonstrate a survival advantage over WASP- negative cells and have the potential to restore adequate immune function and platelet count.

2.4.2. Pharmacology

2.4.2.1. Primary pharmacodynamic studies

The applicant presented multiple studies from literature (from 2005 to 2011) and submitted data of *in vivo* and *in vitro* studies.

Studies showed that vectors induced WASP expression in dendritic cells, with transduced cells forming podosomes similar to healthy individuals, indicating that lentiviral vectors transduced T cells efficiently, with cytokine stimulation improving transduction rates leading to functional correction with no hematopoietic toxicity.

It is also noted that vectors containing the WASP promoter provided higher WASP expression compared to vector containing the PGK promoter.

It was also shown that the presence of either wild-type or mutant WPRE elements enhanced transgene expression without affecting viral production and both WPRE versions restored function in mice.

The GLP *in vitro* study showed that CD34+ cells from WAS patients had a lower growth rate compared to those from healthy controls. While clonogenic capacity was similar between protocols, WAS patient cells had fewer colony forming units (CFUs). The 2-hit protocol resulted in higher vector copy numbers and better gene transfer in WAS patient cells, suggesting it may be the optimal approach for gene therapy, although conclusive statements on transduction efficiency were not provided.

In vivo, murine surrogate test item transduced ex vivo with WAS LVV and infused into mice models. Two different mice WAS disease models were used as proof of concept (POC). The two models were: a WAS knockout (WKO) mouse on a C57BL/6 background, which required sublethal irradiation conditioning for transduced cell engraftment and a WKO mouse on SV129 background that required lethal irradiation conditioning.

Results showed a stable engraftment of WASP-expressing hematopoietic cells was observed after 2 to 4 months in blood, bone marrow and spleen, with representation across T cell, B cell, granulocyte and myeloid lineages. High levels of engraftment were still observed 12 months after a single infusion of WAS LVV-transduced Lin- cells into WKO mice and showed restoration of immune functions.

Moreover, transduction of CD34+ HSPCs with the clinical WAS LVV construct (w1.6W_mut6) did not interfere with the ability of these cells to differentiate into the megakaryocyte phenotype and expression of the WAS transgene was retained after differentiation into the megakaryocytic lineage.

These pharmacological studies demonstrate the feasibility and efficacy of ex-vivo gene therapy with lentiviral vectors in vitro and in vivo in WKO mice, showing partial restoration of cellular functions such as podosome formation, T-cell proliferation and improvement of immune colitis symptoms.

2.4.2.2. Secondary pharmacodynamic studies

No standalone secondary pharmacodynamic studies have been conducted by the applicant but literature data are presented (Toscano 2008).

The study examined the effects of overexpressing the eGFP-WASp protein in different cells. The results show that, at low levels of eGFP-WASp expression, the normal formation of actin filaments is preserved. However, at higher levels of eGFP-WASp, actin filament organization is disrupted, with WASp clusters forming that are no longer associated with actin. This indicated that overexpression might affect the cells' morphology and function (cytotoxicity. According to literature, it can be postulated that while WASp overexpression may help WAS-deficient cells, its expression is more tightly controlled in non-WAS-deficient cells. Further, it seems that hematopoietic cells (like Jurkat cells) have internal mechanisms that regulate WASp expression to prevent toxicity.

Transcriptionally targeted lentiviral vectors, such as the WEW vector, which uses a tissue-specific promoter, allow safe and effective WASp expression in hematopoietic cells without toxic effects. Of note, the WEW vector was generated by replacing the SFFV promoter in the SEW vector with a 500-bp fragment of the WAS proximal promoter in the WW vector (Martin et al., 2005).

2.4.2.3. Safety pharmacology programme

No standalone safety pharmacology studies have been conducted. Since the expression of WASP is under the control of the WAS promoter and regulated by the co-expression of WIP, accidental expression in non-target cells (such as heart cells) is unlikely.

2.4.2.4. Pharmacodynamic drug interactions

No data have been submitted in this application.

2.4.3. Pharmacokinetics

A study evaluating product cell fate was conducted including assessment of survival/engraftment, biodistribution and cellular differentiation.

Due to the nature of the drug product, only hematopoietic compartments were evaluated in addition to brain and testis. Healthy donor HSPCs were used and the immunodeficient recombination-activating gene 2 (Rag2)-/-IL2r-gamma chain-/- (Rag2-/-IL2r-γc-/-) neonatal mouse was used to avoid immunogenicity. No human or LVV sequences were detected in non-hematopoietic tissues (brain and testes), confirming that the WAS LVV-transduced HSPCs distributed to hematopoietic tissues, as expected, and not to non-hematopoietic tissues. In addition, no evidence of vector sequences in bystander murine cells in hematopoietic tissues was found.

2.4.4. Toxicology

2.4.4.1. Single dose toxicity

The ability of WAS LVV-transduced HSPCs to exhibit normal clonogenic potential was assessed in several experiments.

The data showed that WAS LV vector can efficiently transduce CD34+ cells from bone marrow (BM) and mobilised peripheral blood (mPB) in Healthy Controls (HCs) and WAS patients without detectable effect on the growth and similar in vitro differentiation of CD34+ cells within the different protocol. A significant effect was observed only considering the source of cells. In particular, WAS patient’s cells displayed a reduced capacity to grow in culture and in clonogenic potential. A higher efficiency in gene transfer was shown in WAS

patients CD34+ as compared to HC cells, possibly due to a higher susceptibility to infection from LV or a cell growth selective advantage of transduced cells over untransduced cells in vitro.

The clonogenic potential (CFU) of human CD34+ cells derived from umbilical cord blood (UCB) following transduction with WAS LVV was also assessed. The data confirmed that the clonogenic potential of CD34+ cells transduced with WAS LVV was maintained. From a plate in the clonogenic assay, single colonies were picked, lysed and analysed by qPCR to determine transduction efficiency mean of VCN was 1.83 ± 1.50, with a mean transduction efficiency of 77.1 ± 14.7.

The transduction method intended for clinical use involves two cycles of transduction. Transduction protocols were compared in in vitro studies and the in vivo serial transplant study, a single-cycle transduction protocol was used for the main in vivo toxicity studies using murine Lin- HSPCs. This was considered suitable as a higher transduction efficiency is achieved with murine HSPCs and only one cycle of transduction was required to obtain a number of integrated LVV copies in Lin- HSPCs equal or superior to the VCN expected to be achieved in the clinical studies.

In-vitro comparability of fresh versus frozen formulation of gene-transduced WAS CD34+ HSPCs was assessed in order to bridge the clinical data obtained with fresh transduced cells to a cryopreserved formulation. Data produced in this study suggest that WAS CD34+ transduced cells during the process of freezing and thawing maintained intact immunophenotype and clonogenic potential and no difference was observed in between the two formulations.

Two different WAS disease models were used to evaluate in vivo safety of transduced HSPCs: a WAS knockout (WKO) mouse on a C57BL/6 background, which required sublethal irradiation conditioning for transduced cell engraftment and a WKO mouse on SV129 background that required lethal irradiation conditioning. In both models, the test article was comprised of a mouse surrogate test article: lineage-deficient (Lin-) HSPCs transduced with either the w1.6W or the w1.6W_mut6 (clinical) vector.

Hematopoietic stem cells from WAS knockout (WKO) mice (Lin- cells purified from the BM) were transduced with the w1.6W LV, at MOI 10, 20 or 200 (1x10^7, 2x10^7 or 2x10^8 TU/ml, respectively).

Either a pre-final version (wtWPRE) or a final version (mutated WPRE) of w1.6W LV was used. The efficacy of the initial transduction in WKO Lin- cells ranged from 33 to 93%. Restoration of WASP expression was observed in WKO Lin- cells after transduction with the w1.6W LV. Following transplantation into sub-lethally irradiated sex-mismatched recipients, engraftment of the donor cells measured in BM and splenic T and B lymphocytes was reproducibly high and ranged from 66 to 100%. Engraftment level was comparable in BM cells and splenic lymphocytes.

The presence of the integrated vector was detected in BM and splenic T and B lymphocytes. Vector copy number in the tested samples ranged from 0.1 to 7.9. Vector copy number was higher in splenic T lymphocytes than in BM cells. The proportion of WASP+ cells was the highest in splenic T cells probably as a result of a selective advantage for WASP+ T cells during maturation. This may also explain the highest vector copy number observed with splenic T lymphocytes compared to BM cells. In all tested animals treated with the w1.6W LV, the capacity of T cells to produce IL-2 was corrected. Accordingly, increase or correction of the proliferative capacity of T cells was also reported in most of the treated animals.

Systemic blood cell count evaluation in the w1.6W LV treated mice failed to show abnormal cellular expansion.

Macroscopic and histologic analysis performed upon sacrifice of the w1.6W LV treated animals did not reveal an overt occurence of tumors. Given the model used, a normal incidence of tumors was reported. Most of the

tumors were non-hematopoietic tumors of host origin. The few hematopoietic tumors observed (n=4) were of host origin and untransduced. Therefore, they are not attributable to a side effect of the gene therapy product. However, the risk of insertional mutagenicity with the integrative viral vector is an established feature, albeit mitigated in that case by the SIN virus and low VCN.

Serial transplantation experiments over a cumulative period of 10 months demonstrated that the VCN in bone marrow and spleen of secondary recipients was comparable to the primary recipients. Multilineage hematopoietic cell reconstitution in all groups was demonstrated.

No macroscopic or microscopic evidence of tumors were observed in secondary recipients of wt GFP and WKO GFP-transduced cells. One secondary recipient belonging to the WA 1X group (8.3%) and two secondary recipients of the WA 2X group (15.4%) developed thymic lymphomas, but molecular analysis confirmed that lymphomas were of host origin and did not contain LVV sequences. In conclusion, results do not reveal any increased mortality, tumorigenicity, or other forms of toxicity related to the transduction of mouse SV129 WKO Lin- HSPCs with w1.6W LVV.

2.4.4.2. Repeat dose toxicity

No repeat-dose toxicity studies have been presented by the applicant.

2.4.4.3. Genotoxicity

Several studies were conducted to evaluate the potential mutagenicity of WAS LVV, including in vitro immortalization (IVIM) experiments and in vitro and in vivo vector insertion site analysis (VISA).

IVIM test

In the IVIM test, lineage negative (Lin-) BM cells were used from untreated wildtype C57BL6/J mice which lack a pre-existing transforming lesion. Cells were pre-stimulated for 2 days prior to transduction for 4 consecutive days with the WAS LVV (RRL.PPT.WASP.WAS.pre). After transduction, cells were expanded in mass culture for 2 weeks then plated onto 96-well pates.

The positive control vector and all gammaretroviral vectors with the Spleen Focus-Forming Virus (SFFV) enhancer–promoter induced re-plating in every experiment. Only the LVV containing the WAS promoter and WAS transgene tested negative in the assay. Although this result is based on a limited number of experimental replicates (n = 4), it suggests that the combination of the endogenous WAS promoter with the LVV backbone may be a factor reducing the vector's immortalization potential. The absence of re-plating activity with the WAS LVV could not be explained by a lower transduction efficiency, as the average VCN in the four assays conducted with WASP LVV were 0.80, 2.52, 3.12 and 5.15 i.e in line with those obtained previously.

VISA test

VISA was evaluated in vitro and in vivo in two separate studies.

In vitro study:

The integration profile of the clinical vector (w1.6W_mut6) was analysed in healthy donor or WAS patient CD34+ cells in vitro and in UCB cells from healthy donors 8 weeks after engraftment in Rag2−/−/γc−/− mice.

The insertion profile in vitro was typical of the LV vectors with significant preference for transcriptional units (from 74.5 to 77.0 % of integrations) as compared to in silico generated random insertions (n=100,000, 40.7%) (P<0.01, z-test for two proportions).

In vivo studies:

The preference for transcriptional units inside genes was maintained in vivo, 8 weeks after transduced cell infusion in Rag2−/−/γc−/− mice. There was no in vivo enrichment of the frequency of insertion sites in close proximity to genes involved in cancer (6.3% in both HD and WAS patients versus 16.1% in random in silico insertions dataset).

In addition, by comparing the insertions in BM and thymus from individual treated mice or in BM, thymus and spleen from a pool of three mice, the presence of shared identical integrant was observed among the different hematopoietic compartments analysed.

In the second study, Integration Site (IS) was evaluated using vector integration tag analysis (VITA) on WAS (w1.6W) LVV-transduced Lin- cells before and after transplantation into irradiated SV129 WKO mice.

Despite a few insertions of the WAS LV into genes previously described as Common Insertion Site(CIS) in the retrovirus-induced tumor database, no obvious bias for the distribution of insertions into specific categories of genes were found.

Finally, a recent publication by (Calabria et al. Nature,vol 636 2024), summarises the molecular properties of lentiviral vectors used for CD34+ cell transduction in three genetic diseases including WAS. The authors confirm, in more than 1,680,000 integrations the features already mentioned, with KDM2A, PACS1, HLA, TNRC6C, SETD2 genes more frequently targeted, with no associated risks identified. Interestingly, the data show the proportion of grafted cells contributing to circulating cells production (0.0003-0.7%). The results indicate that, it may be barely detectable to anticipate clonal dominance or early detection of a transformation event using current qPCR-based methods, unless a selective advantage is conferred to transduced cells, which then would induce a higher proportion of circulating cells.

In conclusion, the analysis of vector integrations showed a strong bias of WAS vector for coding regions without major preference for regions upstream of transcription start sites.

2.4.4.4. Carcinogenicity

While, standard carcinogenicity studies are usually not required due to the nature of the advanced medicinal product, the tumorigenic potential was investigated in the general toxicology biosafety study and in transplantation of WAS LVV-transduced BM cells from SV129-WKO mice into secondary recipients. Overall, these studies demonstrated no increased tumorigenic potential over a period of up to 16 months when WAS LVV-transduced HSPCs were transplanted into WAS deficient mice.

2.4.4.5. Reproductive and developmental toxicity

In sub-lethally irradiated Rag2-/- Il2r-gamma chain-/- neonatal male and female mice injected intravenously with Telethon003 (0.3 x 106 cells per animal), transduced cells distributed primarily to hematopoietic tissues with no detection of vector signal in testes. This shows that the risk of germline transmission from male patients is considered low and further studies are not considered needed. More generally, available data are supportive of an absence of risk for insertion in germ cells following ex vivo gene correction using vectors.

This applies to both sex. However, no studies on female animal are required considering that this is a treatment of a X-linked genetic disease present only in male patients.

In addition, there was no treatment-related effect on male or female reproductive organs in the mouse toxicity study conducted with a murine surrogate (one case of ovary germinal cell tumour is stated to be of host origin).

2.4.4.6. Toxicokinetic data

Not applicable.

2.4.4.7. Tolerance

No local tolerance studies were conducted with Telethon003 which is endorsed.

2.4.4.8. Other toxicity studies

No dedicated antigenicity and immunotoxicity studies were performed. The absence of dedicated studies is endorsed. The rationale for not performing toxicity studies testing metabolites and impurity studies is also agreed due to the nature of the medicinal product.

Dependency studies were not performed which is endorsed since there is no known role of WAS in dependency.

To assess the extent of vector shedding, Human BM or UCB derived CD34+ cells transduced with WASP LV were co-cultured at 10:1 and 20:1 ratio with an immortalized cell line permissive to LV transduction (293T) following a 2-hour incubation with medium added of human AB serum, medium without serum or with heat inactivated human AB serum.

Persistence of LV particles on transduced HSPC surface is responsible for secondary transduction proportionally to the HSPC:293T ratio with a mean VCN 0.15-0.20 which is significant lower than both 293T cells and HSPCs directly transduced with WASP LV.

Incubation with human AB serum strongly reduced the secondary transduction of 293T cells (mean VCN 0.01 and 0.02 at 10:1 and 20:1 ratio, respectively; p values=0.0457). Data suggest that in a clinical setting, in which transduced cells are infused into the bloodstream of patients, the potential occurrence of vector shedding and secondary transduction events may be reduced to negligible levels.

In addition, secondary transduction was evaluated in vivo in the biodistribution study (Report 2017N340701). That study confirmed that the vector co-distributed with the human transduced cells and that the LV vector genome remained integrated in the human genome in stable proportions, without showing indication of mobilization or bystander transduction of mouse cells.
In order to exclude generation of replication-competent recombinant viral particles (RCL: Replication-Competent Lentivirus), gag p24 HIV protein was measured in the plasma of mice. All animals tested were negative with the exception of one mouse in the group WAS LVV that showed low level of protein. More detailed molecular analyses were performed both on cells archived from this mice and cells cultured starting from infused WAS LVV, indicating the presence of gagpol packaging plasmid sequences and absence of HIV LTR sequences in the mouse BM in the absence of RCL. These findings suggested the occurrence of rare plasmid integration as a consequence of packaging plasmid carry-over in the LV vector used for transduction.

No evidence of vector integrated in murine cells was reported by B2-SINE PCR even in the mouse found positive for p24.
Based on the observed findings in treated mice and considering the number of cells injected per mouse and the level of p24 expression in the plasma, the risk of plasmid carry-over during the clinical transduction protocol, would fall well below the detection threshold in a patient.

2.4.5. Toxicokinetic Ecotoxicity/environmental risk assessment

Waskyra is administered under controlled contained-use conditions. Blood, organs, cells and tissues donations are prohibited for patients who have received this medication. The recommendations for medical staff are acceptable and recommend wearing of personal equipment and use of disinfectants in the event of contamination.
The risk of dissemination of replication-competent recombinant viral particles is considered negligible. The risk of dissemination of residual WAS LVV particles into the environment is also considered negligible.

Overall, based on the environmental risk assessment provided and following consultation with the relevant GMO competent authorities, it can be agreed that the environmental risk associated with Waskyra are considered negligible.

2.4.6. Discussion on non-clinical aspects

Telethon003 (Telethon003 is also referred as Waskyra in the report) is a gene therapy medicinal product.

The pharmacology, toxicology and genotoxicity of Waskyra were evaluated in vitro and in vivo. The models used were considered acceptable as surrogate of HPSC transduced with Waskyra.

No standalone safety pharmacology studies nor pharmacodynamic drug interactions have been conducted which is acceptable due to the nature of the product consisting of the patient's autologous bone marrow (BM) CD34+ hematopoietic stem cells, transduced ex vivo with a lentiviral vector (LV) carrying the cDNA encoding the Wiskott-Aldrich Syndrome (WAS) gene.

Similarly, standard toxicological assessment was not applicable. Conventional mutagenicity, carcinogenicity and reproductive and developmental toxicity studies have not been conducted, which is in accordance with scientific and regulatory recommendations related to gene therapies.

No repeat-dose toxicity studies have been presented by the applicant, which is acceptable considering that the product is intended to be administered to patients as a single administration, with subsequent engraftment into the patient.

Further, no dedicated antigenicity and immunotoxicity studies were performed. The absence of dedicated studies is endorsed. The rationale for not performing toxicity studies testing metabolites and impurity studies is also agreed due to the nature of the medicinal product.

Dependency studies were also not performed which is endorsed since there is no known role of WAS in dependency.

Pharmacology

The initial studies were conducted using a pre-final version of the WAS lentiviral vector (LVV). Therefore, the *in vitro* data collected using the initial vector were considered representative of the data obtained with the final clinical vector.

The results from the pharmacological studies demonstrate the feasibility and efficacy of ex-vivo gene therapy using lentiviral vectors in WKO mice, with partial restoration of critical immune functions such as podosome formation, T-cell proliferation and the improvement of immune colitis symptoms. In the study on WPRE elements, both wild-type and mutant versions (mut6) were shown to enhance viral expression without impacting viral production and both were equally effective in restoring immune function, including podosome formation in dendritic cells, thymic and B-cell reconstitution and improvement of autoimmune colitis in mice.

In most instances, the transcriptional activity of the w0.5W and w1.6W promoters has been shown to be weaker than that of other internal promoters commonly used in lentiviral vectors, such as EF1α and PGK.

The additional advantage of the longer 1.6 kb promoter is that it contains additional cis acting sequences reputed to be involved in the physiological regulation of WAS expression in T cells and myeloid cells and has therefore been selected in the final construction of the vector.

The long term follow up in vivo study in mice revealed that WASP expression was successfully restored in immune cells, resulting in improved blood cell counts, particularly B cells, platelets and granulocytes after 4 to 16 months follow-up.

This was shown by the stronger transgene expression from the WAS promoter observed in the haematopoietic cell lines (Jurkat, JY, HEL, U937) compared to non-haematopoietic cell lines (HeLa, NIH373, 293T). This preferential expression in haematopoietic cells was more evident in the T cell line (Jurkat) and the erythroid cell lines (HEL) than in the macrophage cell line (U937) and the B cell line (JY). However, it is also noted that transcriptional expression of WAS mRNA was demonstrated in both haematopoietic and non-haematopoietic cells.

The GLP study also revealed that WAS patient cells had lower growth and clonogenic capacity compared to healthy controls. The 2-hit transduction protocol resulted in higher vector copy numbers and better gene transfer efficiency in WAS patient cells. Literature data also supports the presence of immunomodulation markers (such as interleukins, etc…) and their functionality (T cells cytotoxic and DC capability to effectively differentiate, mature and induce antigen-specific T cell activation).

In addition, a study using the SEW vector (SFFV-eGFP-WAS) examined the effects of overexpressing the eGFP-WASp protein. The result indicated that overexpression of WASp might affect cellular morphology and function. while overexpressing WASp may help restore function in WAS-deficient cells. Literature suggests that the absolute levels of WASP are tightly regulated by its binding partner WIP (WASP Interacting Protein) and that the promoter used (1.6 kb WAS) is not expected to provide overexpression. This is endorsed by CAT/CHMP.

The biodistribution study demonstrated that CD34+ HSPCs transduced with the final clinical vector (w1.6W_mut6, WAS LVV) successfully repopulated hematopoietic tissues, including bone marrow, spleen, thymus and liver. These cells showed multilineage differentiation and similar engraftment compared to HSPCs. Terminally differentiated hematopoietic progeny maintained similar vector copy numbers to the original transduced cells, confirming long-term repopulation and multilineage potential.

Importantly, WAS LVV sequences were not found in non-hematopoietic tissues or in by stander mouse cells within hematopoietic tissues, indicating targeted distribution to hematopoietic tissues only.

Assessment of clonogenic potential

The ability of WAS LVV-transduced HSPCs to exhibit normal clonogenic potential was assessed in several experiments. WAS LV vector could efficiently transduce CD34+ cells from BM and MPB in HCs and WAS

patients without detectable effect on the growth and similar in vitro differentiation of CD34+ cells within the different protocols. The transduction method intended for clinical use involves two cycles of transduction. WAS patient's cells displayed a reduced capacity to grow in culture and in clonogenic potential possibly due to a higher susceptibility to infection from LV or a cell growth selective advantage of transduced cells over untransduced cells in vitro. The data confirmed that the clonogenic potential of CD34+ derived from UCB transduced with WAS LVV (2-hits at a MOI of 100) was maintained with a mean VCN of 1.83 ± 1.50 and a mean transduction efficiency of 77.1 ± 14.7.

Drug product formulations

In-vitro comparability of fresh versus frozen formulation of gene-transduced WAS CD34+ HSPCs was assessed in order to bridge the clinical data obtained with fresh transduced cells to a cryopreserved formulation. Data suggest that WAS CD34+ transduced cells during the process of freezing and thawing maintained intact immunophenotype and clonogenic potential and no difference was observed in between the two formulations.

Toxicity

No standalone, GLP *in vivo* pivotal toxicology study has been performed.

The impossibility of using autologous human cells in animal models limits the scope of possible nonclinical studies. Thus, two different WAS disease models were used to evaluate in vivo safety of transduced HSPCs: a WAS knockout (WKO) mouse on a C57BL/6 background, which required sublethal irradiation conditioning for transduced cell engraftment and a WKO mouse on SV129 background that required lethal irradiation conditioning. In both models, the test article was a lineage-deficient (Lin-) HSPCs transduced with either the w1.6W or the w1.6W_mut6 (clinical) vector.

Lin- HSPCs purified from the BM were transduced with the WAS LV, at MOI 10, 20 or 200. The efficacy of the initial transduction in WKO Lin- cells ranged from 33 to 93% with a restoration of WASP expression. The VCN in the tested samples ranged from 0.1 to 7.9. with a higher value in splenic T lymphocytes than in BM cells. The proportion of WASP+ cells was the highest in splenic T cells probably as a result of a selective advantage for WASP+ T cells during maturation.

Macroscopic and histologic analysis performed upon sacrifice of the w1.6W LV treated animals did not reveal an abnormal cellular expansion or overt occurrence of tumors. Given the model used, a normal incidence of tumors was reported. Most of the tumors were non-hematopoietic tumors of host origin. However, the risk of insertional mutagenicity with the integrative viral vector is an established feature, albeit mitigated in that case by the SIN and low VCN.

Serial transplantation experiments over a cumulative period of 10 months demonstrated that the VCN in bone marrow and spleen of secondary recipients was comparable to the primary recipients and multilineage hematopoietic cell reconstitution in all groups was demonstrated.

One secondary recipient belonging to the WAS LVV group 1X (8.3%) and two secondary recipients of the WAS LVV 2X group (15.4%) developed thymic lymphomas but molecular analysis confirmed that all were of host origin and did not contain LVV sequences. In conclusion, results do not reveal any increased mortality, tumorigenicity, or other forms of toxicity related to the transduction of SV129 WKO Lin- HSPCs with WAS LVV.

Mutagenicity

Several studies were conducted to evaluate the potential mutagenicity of WAS LVV, including in vitro immortalization (IVIM) and in vitro/in vivo vector insertion site analysis (VISA).

In the IVIM test, Lin- HSPCs were used from untreated wild type C57BL6/J mice which lack a pre-existing transforming lesion. The positive control vector and all gammaretroviral vectors with the SFFV enhancer–promoter induced re-plating in every experiment. Only the LVV with the WAS promoter and WAS transgene was negative in the assay. Although fewer experimental replicates were conducted (n=4), the use of the endogenous WAS promoter in combination with the LVV backbone was therefore considered a factor in reducing the immortalization potential of the vector.

The absence of re-plating activity with the WAS LVV could not be explained by a lower transduction efficiency, as the average VCN in the four assays conducted with WAS LVV were 0.80, 2.52, 3.12 and 5.15 i.e. in line with those obtained previously.

The insertion profile in vitro showed significant preference for transcriptional units inside genes (from 74.5 to 77.0 % of integrations) as compared to in silico generated random insertions (n=100,000, 40.7%) ($P<0.01$, z-test for two proportions). After 8 weeks from transduced cell infusion in Rag2−/−/γc−/− mice, there was no in vivo enrichment of the frequency of insertion sites in close proximity to genes involved in cancer (6.3% in both HD and WAS patients versus 16.1% in random in silico insertions dataset). In addition, the presence of shared identical integrant was observed among the different hematopoietic compartments analysed.

In a second study, IS was evaluated using vector integration tag analysis (VITA) on WAS LVV-transduced Lin- cells before and after transplantation into irradiated SV129 WKO mice. No obvious bias for the distribution of insertions into specific categories of genes were found.

Finally, a recent publication by Andrea Calabria et al. in Nature, Vol 636, 5 December 2024, summarises the molecular properties of lentiviral vectors used for CD34+ cell transduction in three genetic diseases including WAS. They confirm, in more than 1,680,000 integrations the features already mentioned, with KDM2A, PACS1, HLA, TNRC6C, SETD2 genes more frequently targeted, with no associated risks identified. Interestingly, the data show the proportion of grafted cells contributing to circulating cells production (0.0003-0.7%). This indicates that, to anticipate clonal dominance or early detection of a transformation event, it might be barely detectable using current qPCR-based methods, unless a selective advantage is conferred to transduced cells, which then would induce a higher proportion of circulating cells.

In conclusion, the analysis of vector integrations showed a strong bias of WAS vector for coding regions without major preference for regions upstream of transcription start sites, a feature typical of lentiviral vector.

For WAS, historical data, with more than 15 years of follow-up using the same type of vector have not identified any particular risk of vector integration. However, due the integrative nature of the viral vector, the risk of insertional mutagenesis requires a 15-year long term follow up of patients as for other genetically modified cell therapies and detection of clonal dominance or transformation in case of cancer occurrence.

Malignancy due to insertional mutagenesis is an important potential risk. This risk will be further characterised in the imposed PASS in the post approval setting which is a Long-term Follow-up Study for subjects Previously Treated with Autologous ex vivo Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome.

Developmental and reproductive toxicity

The absence of standard developmental and reproductive toxicity studies with Telethon003 is acceptable in view of the patient population, nature of the gene therapy, and preconditioning regimen with developmental toxicants. Available data show a low risk of germline transmission from male patients.

Immunogenicity

WAS-LV-transduced HSPCs are not anticipated to induce an immune response. Therefore, dedicated antigenicity and immunotoxicity non-clinical studies are not warranted.

Vector shedding

To assess the extent of vector shedding, human BM or UCB derived CD34+ cells transduced with WAS LVV were co-cultured at 10:1 and 20:1 ratio with an immortalized cell line permissive to LV transduction (293T) with medium added of human AB serum. Persistence of LV particles on transduced HSPC surface which is responsible for secondary transduction was shown to be low (mean VCN 0.15-0.20) and incubation with human AB serum strongly reduced the secondary transduction of 293T cells. It is considered that in clinical setting, in which transduced cells are infused into the bloodstream of patients, the potential occurrence of vector shedding and secondary transduction events would be reduced to negligible levels.

Plasmid integration

In order to exclude generation of RCL, gag p24 HIV protein was measured in the plasma of mice. All animals tested were negative with the exception of one mouse that presented gagpol packaging plasmid sequences but not HIV LTR sequences in the BM in the absence of RCL. These findings suggested the occurrence of rare plasmid integration as a consequence of packaging plasmid carry-over in the LV vector used for transduction. No evidence of vector integrated in murine cells was reported by B2-SINE PCR even in the mouse found positive for p24.

Based on the observed findings in treated mice and considering the number of cells injected per mouse and the level of p24 expression in the plasma, the risk of plasmid carry-over during the clinical transduction protocol, would fall well below the detection threshold in a patient. Therefore, the risk of plasmid integration is considered negligeable.

Finally, the environmental risk associated with Waskyra are considered negligible based on the available data and the documents received.

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

2.4.7. Conclusion on the non-clinical aspects

In conclusion, it is considered that the potential occurrence of vector shedding and secondary transduction events and risk of plasmid integration are negligible and have been adequately addressed.

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

Malignancy due to insertional mutagenesis is an important potential risk. This risk will be further characterised in the imposed PASS in the post approval setting which is a Long-term Follow-up Study for subjects Previously Treated with Autologous ex vivo Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome.

From a non-clinical perspective, non-clinical data are sufficient and support a marketing authorisation.

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

2.5. Clinical aspects

2.5.1. Introduction

GCP aspects

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.

Table 1. Tabular overview of clinical studies

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
TIGET-WAS (201228)	8 enrolled, 8 treated	Phase I/II, open-label, single-arm, non-randomized, prospective, single-center study using the 12-month pre-treatment period as comparator	Telethon 003. IV Infusion, single dose. Cell source: BM (n=5), mPB (n=2) BP/mPB (n=1)	> 6 months of age Inclusion : - Diagnosis of WAS defined by genetic mutation, AND severe clinical score OR severe WAS mutation or absent WASP - no HLA identical donor and negative search for matched unrelated donor - signed informed consent Exclusion - HIV positive - end-organ dysfunction - neoplasia - myelodisplastic syndrome/ myeloid leukemia - prior allogenic HSCT
OTL-103-4	10 enrolled, 10 treated	Phase III, open-label, single-arm, non-randomized, multi-centre study using the 12-month pre-treatment period as comparator	Telethon 003. IV Infusion, single dose. Cryopreserved formulation Cell source: fresh mPB (n=10)	> 6 months of age Inclusion : - Diagnosis of WAS defined by genetic mutation, AND severe clinical score OR severe WAS mutation OR absent WASP - no HLA identical donor for HSCT - signed informed consent Exclusion - end-organ dysfunction - malignant neoplasia

				- myelodysplasia, myelodisplastic syndrome/ myeloid leukemia - prior allogenic HSCT - HIV infection - previous GT
Expanded access program (EAP), HE and CUP	10 enrolled, 9 treated	Prospective single centre treatment program (compassionate)	Telethon 003. IV Infusion, single dose. Cryopreserved formulation Cell source: fresh mPB (n=10)	> 6 months of age Inclusion : - Diagnosis of WAS defined by genetic mutation - severe clinical score - no HLA identical donor for HSCT OR age > 5 years - signed informed consent Exclusion - positive for HIV infection - malignant neoplasia - myelodysplasia, myelodisplastic syndrome/ myeloid leukemia - end-organ dysfunction - prior allogenic HSCT / previous GT

2.5.2. Clinical pharmacology

There are no human pharmacology data as such due to the nature of the medicinal product. Please refer to the efficacy section for the relevant "pharmacodynamic" data related to engraftment and related endpoints.

2.5.2.1. Pharmacokinetics

Conventional studies on pharmacokinetics, absorption, metabolism, and elimination are not applicable for this gene therapy product. This is in line with "Guideline on quality, non-clinical, and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/67139/2008 Rev1-corr)".

No biodistribution studies were conducted in humans. Biodistribution and persistence of genetically modified cells were addressed through non-clinical and clinical endpoints.

In the Telethon003 clinical development program, participants received pre-conditioning with rituximab on day -22 days (±1 day) before administration of Telethon003 at a single dose of 375 mg/m^2 and a reduced intensive conditioning (RIC) regimen consisting of IV busulfan at a target AUC of 36,000-48,000 ng/ml*h guided by PK monitoring and fludarabine (total daily dose 60 mg/m^2).

In study OTL-103-4, while the correct dosing and pharmacokinetic monitoring was performed, 5 patients had significantly higher exposures to busulfan than the target and presented multiple serious adverse events.

2.5.2.2. Pharmacodynamics

Mechanism of action

Telethon003 is a gene therapy product in which a correct copy of the WAS gene is stably inserted in the genome of the participants' HSPCs, thereby correcting the genetic defect in these cells. The progeny of these gene-corrected stem cells forms the full array of hematopoietic cells, which therefore also carry the corrected

gene and express WASP that is required for the normal function of the hematopoietic cells. No dedicated clinical pharmacology studies were performed.

The mechanism of action was demonstrated by the efficacy endpoints, i.e. engraftment of gene-corrected cells, WAS protein expression, T cell function described below.

PD biomarkers

A range of doses and various pharmacodynamic parameters were measured in the clinical studies.

The applicant has submitted the validation reports for several biomarker assays: the assays for determination of VCN as a measure of engraftment efficiency by digital droplet PCR (ddPCR), the WAS protein expression by flow cytometry. The qualitative detection of anti-WASP antibodies has also been performed.

Primary and Secondary pharmacology

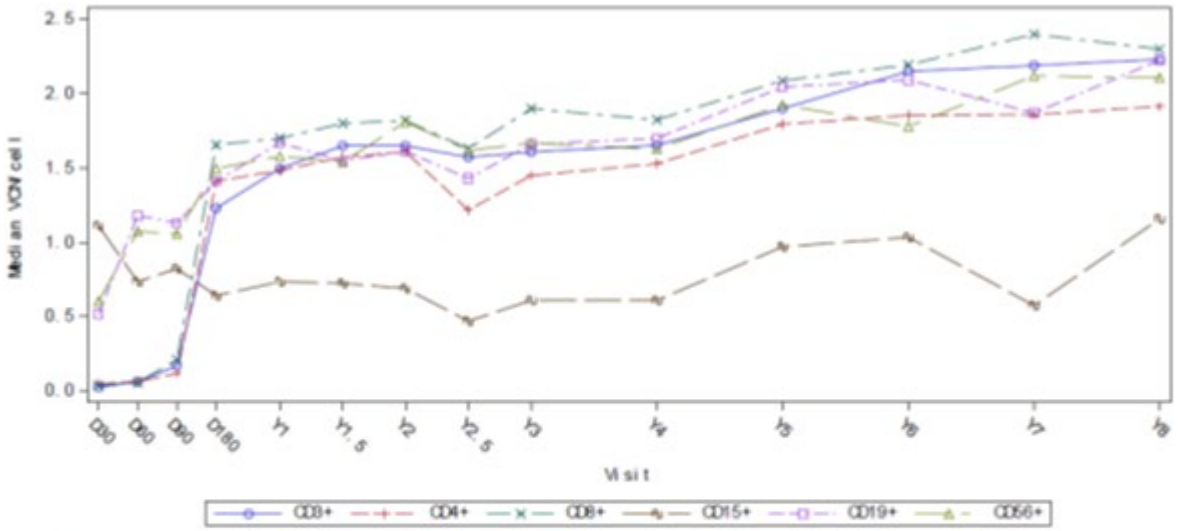
There are no human pharmacology data as such due to the nature of the medicinal product.

Engraftment of gene corrected cells

All participants in the efficacy population achieved adequate engraftment of BM CD34+ and/or PB CD3+ cells, defined as ≥0.04 VCN/cell in BM CD34+ cells (equivalent to 4% of cells being genetically corrected assuming a VCN of 1) or ≥0.1 VCN/cell in BM CD3+ cells (equivalent to 10% of cells being gene corrected assuming a VCN of 1).

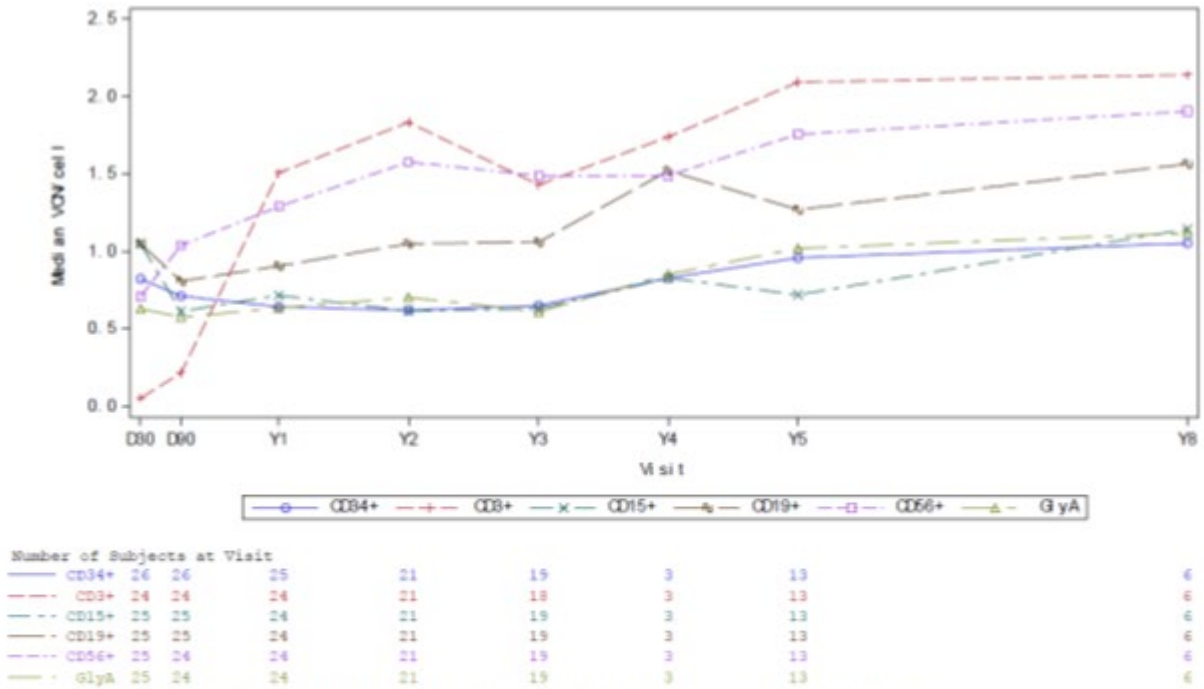
VCN/cell in mPB and BM cell lineages

Figure 3. Median values for VCN/cell in peripheral blood cell lineages (Efficacy population)



D=Day; CD=cluster of differentiation; VCN=vector copy number; Y=Year
Results based on fewer than two participants were omitted.
Source: Figure 2.7.3.3.1.11

Figure 4. Median values for VCN/cell in bone marrow cell lineages (Efficacy population)



D=Day; CD=cluster of differentiation; GlyA=glycophorin A; VCN=vector copy number; Y=Year
Results based on fewer than two participants were omitted.
Source: Figure 2.7.3.3.1.10

The threshold for VCN/cell was based on observations from a GT study for severe combined immunodeficiency due to adenosine deaminase deficiency. Results were well above threshold. No correlation dose/VCN was established (see below).

The percentage of LVV-transduced CFU in BM cells (%LVV+ BM-CFU) was around 50% in all participants evaluable at Years 1 and 2 that remained high up to year 8.

Table 2. Summary of percentage of lentiviral vector transduced-colony forming units in bone marrow cells (Efficacy population)

%LVV+ BM-CFU	Day 30	Day 90	Year 1	Year 2	Year 3	Year 5	Year 8
n	23	23	23	21	16	10	6
Median	51.00	51.00	49.00	48.10	39.45	50.50	66.50
Min, Max	18–77	12.5–81.0	17.0–85.0	20.7–86.0	10.0-91.0	15.0-100.0	30.0-100.0

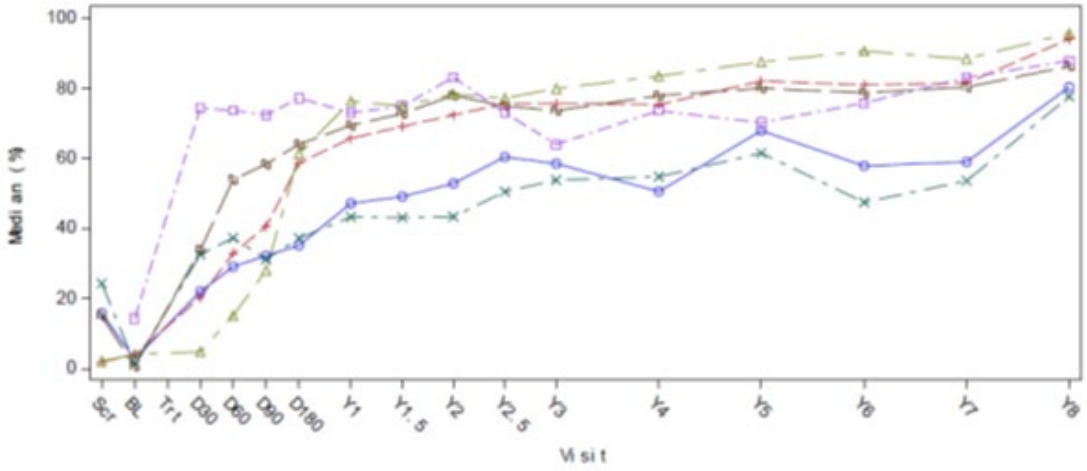
LVV=lentiviral vector; CFU=colony forming units; BM=bone marrow; min=minimum; max=maximum
If there was more than 1 value within an assessment window, the earliest available measurement for that visit was used in the summary.
Source: Table 2.7.3.3.1.7

WASP expression

The presence of WASP was assessed using an intracellular staining with a WASP-specific antibody and the proportion of cells expressing WASP was quantified at regular time intervals post-treatment. Specific hematopoietic cell surface markers were used simultaneously to detect transgene expression in individual lymphoid and myeloid cell lineages.

The median percentage of platelet and lymphocyte cells expressing WASP increased steadily up to 6 months compared to baseline levels and remained high afterwards (from 14% at baseline to 77% at D180 for platelets and respectively from 4% at baseline to 59% at D180 for lymphocytes). The median percentage of platelets expressing WASP increased rapidly whereas the increase in lymphocytes was more gradual.

Figure 5. Median values of peripheral blood cells expressing WASP Assessed by flow cytometry over time (efficacy population)



Number of Subjects at Visit															
WASP/B Cells (%)	2	24	18	23	23	22	24	21	20	13	18	20	15	12	9	6
WASP/Lymphocytes (%)	3	26	24	25	23	22	23	21	20	13	19	20	16	12	9	6
WASP/Monocytes (%)	2	24	21	22	23	23	23	21	20	13	19	20	16	12	9	6
WASP/NK Cells (%)	2	25	23	24	24	23	24	21	20	13	19	20	16	12	9	6
WASP/Platelets (%)	1	23	17	19	20	20	20	19	20	10	19	19	15	12	7	4
WASP/T Cells (%)	2	25	23	25	24	23	24	21	20	13	19	20	16	12	9	6

BL=Baseline; D=Day; NK=natural killer; Scr=Screening; Trt=Treatment (Telethon003); WASP=Wiskott-Aldrich syndrome protein; Y=Year.
Transduced cells infusion (gene therapy) was performed at the Treatment Visit (Trt).
Results based on fewer than two participants are omitted.
Participants enrolled at the Children's Healthcare of Atlanta site do not have WASP expression assessed in platelets.

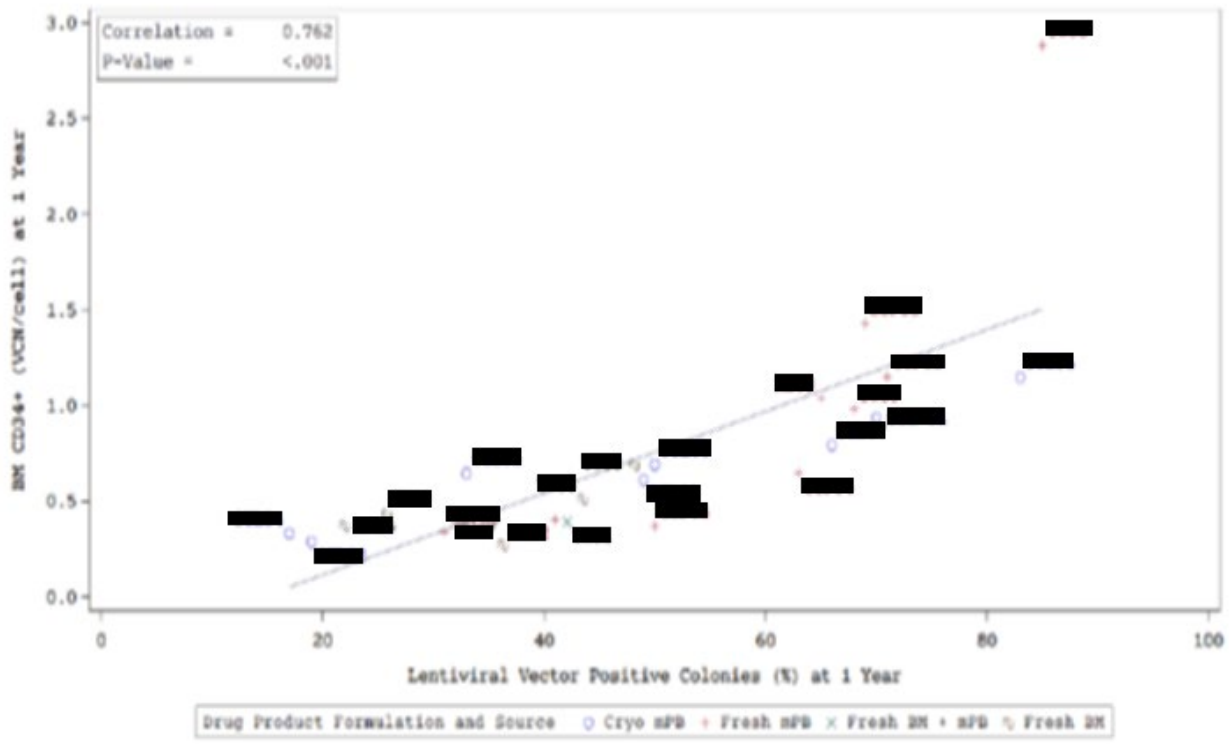
T cell function

The T cell response is associated with functional WASP expression. All participants had reduced proliferative responses to CD3 stimulation at baseline but showed increased proliferative potential in vitro over time. Responses to CD3 stimulation increased over time for all tested doses, with participant T-cells showing the greatest proliferative response at Year 8 when stimulated with the higher dose of anti-CD3i antibody (1 µg/mL). At this stimulation dose (1 µg/mL), the median proliferative response was within normal range as from Year 1.

Correlation between chimerism and engraftment and efficacy

A strong positive correlation was shown between the level of gene corrected HSPCs chimerism (%LVV+ colonies) in BM at 1 year and the level of gene correction in BM compartment (VCN/cell in BM CD34+) at 1 year, between % LVV+ and platelet count ($p<.01$), and between %LVV+ and PB WASP+ cells (percentage of platelets) at Year 2 ($p<.01$).

Figure 6. Correlation of chimerism (%LVV) and engraftment of bone marrow CD34 (VCN/cell) at Year 1 (efficacy population)



%LVV=percentage lentiviral vector; BM=bone marrow; CD=cluster of differentiation; cryo=cryopreserved; mPB=mobilized peripheral blood; VCN=vector copy number
Abbreviated participant IDs were used to label data points.

In terms of stem cell source (BM vs mPB), the VCN/cell in BM CD34+ cells and PB CD3+ T-cells were comparable between BM participants and mPB participants and the levels of WASP expression were comparable.

In addition, the type of drug product formulation (fresh vs cryopreserved) did not affect the engraftment efficiency or WASP expression. Overall, the different drug product attributes used in drug development had no impact on the level of engraftment.

2.5.3. Discussion on clinical pharmacology

Pharmacokinetics

Conventional studies on pharmacokinetics, absorption, metabolism, and elimination are not applicable for this gene therapy product in line with "Guideline on quality, non-clinical, and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/67139/2008 Rev1-corr)".

No biodistribution studies were conducted in humans. Biodistribution and persistence of genetically modified cells were addressed through non-clinical and clinical endpoints.

Pharmacodynamics

Telethon003 is a GT product in which a correct copy of the WAS gene is stably inserted in the genome of the participants' HSPCs, thereby correcting the genetic defect in these cells. The progeny of these gene-corrected stem cells forms the full array of hematopoietic cells, which therefore also carry the corrected gene and express WASP that is required for the normal function of the hematopoietic cells. No dedicated clinical pharmacology studies were performed. This is acceptable due to the nature of the medicinal product.

The mechanism of action is demonstrated by the efficacy endpoints, i.e. engraftment of gene-corrected cells, WAS protein expression, T cell function.

A range of doses and various pharmacodynamic parameters were measured in the clinical studies.

The applicant has submitted the validation reports for several biomarker assays, together with an overview of assay used for the evaluation of clinical outcome. The assays for determination of VCN by ddPCR and WAS protein expression by flow cytometry are considered the most important, as they support the primary endpoints in phase 3 study WAS-104-3. The assays are considered suitable for the intended use.

Method transfer was done by comparing the VCN from genomic DNA purified from peripheral blood and bone marrow (BM) tissues and DNA from cell lysate of BM derived colonies of gene therapy patients. The results showed for the genomic DNA that the VCN number had acceptable levels of difference between the two assays. For the colony forming cells, there was a large difference in the variability of the VCN for these samples between the two methods. The applicant considered the discrepancies where VCN values were greater than 30% CV between the two methods as not relevant, as ultimately the engraftment were found to be similar. This is agreed by CAT/CHMP.

Primary and Secondary pharmacology

Engraftment of gene corrected cells

All participants in the efficacy population achieved adequate engraftment of BM CD34+ and/or PB CD3+ cells, defined as ≥0.04 VCN/cell in BM CD34+ cells (equivalent to 4% of cells being genetically corrected assuming a VCN of 1) or ≥0.1 VCN/cell in BM CD3+ cells (equivalent to 10% of cells being gene corrected assuming a VCN of 1.

The applicant considered that a threshold of VCN/cell of 1 would be sufficient for the therapeutic effect. This threshold for VCN/cell is based on the observations from a GT study for severe combined immunodeficiency due to adenosine deaminase deficiency. The CAT/CHMP considered this threshold remains mostly empirical and that the disease to be corrected might influence the level of requirement on VCN. However, as results were well above the proposed threshold and that no correlation dose/VCN was established, this was considered acceptable by CAT/CHMP

The percentage of LVV-transduced CFU in BM cells (%LVV+ BM-CFU) was around 50% in all participants evaluable at Years 1 and 2 that remained high up to year 8, however with a wide range (15-100%).

WASP expression

The presence of WASP was assessed using an intracellular staining with a WASP-specific antibody and the proportion of cells expressing WASP was quantified at regular time intervals post-treatment.

The median percentage of platelet and lymphocyte cells expressing WASP increased steadily up to 6 months compared to baseline levels and remained high afterwards (from 14% at baseline to 77% at D180 for platelets and respectively from 4% at baseline to 59% at D180 for lymphocytes).

T cell function

The T cell response is associated with functional WASP expression. All participants had reduced proliferative responses to CD3 stimulation at baseline but showed increased proliferative potential in vitro over time. Responses to CD3 stimulation increased over time for all tested doses.

Correlation between chimerism and engraftment and efficacy

A strong positive correlation was shown between the level of gene corrected HSPCs chimerism (%LVV+ colonies) in BM at 1 year and the level of gene correction in BM compartment (VCN/cell in BM CD34+) at 1 year, between % LVV+ and platelet count ($p<.01$), and between %LVV+ and PB WASP+ cells (percentage of platelets) at Year 2 ($p<.01$).

In terms of stem cell source (BM vs mPB) and type of formulation (fresh and cryopreserved), the VCN/cell in BM CD34+ cells and PB CD3+ T-cells and the levels of WASP expression were comparable. Overall no correlation between drug product attributes and engraftment was found.

Pharmacodynamics interactions

No formal interactions studies have been performed, since this a gene therapy product and drug-drug interactions are not expected.

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

2.5.4. Conclusions on clinical pharmacology

Absence of standard PK information is considered acceptable for a GT product. The applicant has submitted the validation reports for the bioanalytical assays used as PD biomarkers, the assays are suitable for the intended use.

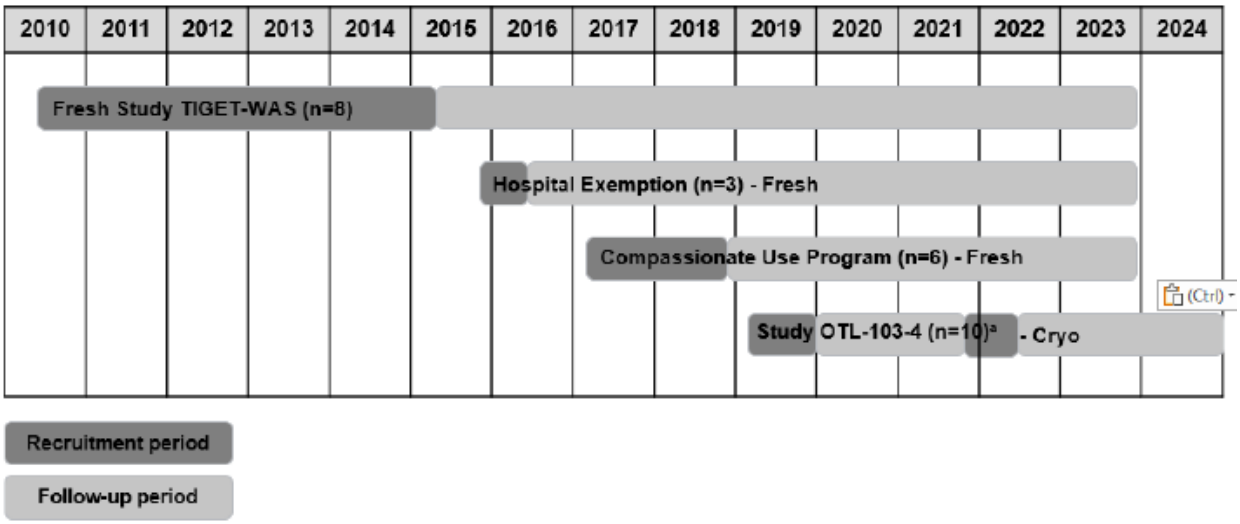
The clinical pharmacology data are sufficient and results acceptable to support the marketing authorisation for Waskyra.

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

2.5.5. Clinical efficacy

Two formulations, fresh and cryopreserved and two sources of starting material were used in clinical development: study TIGET-WAS used the fresh formulation and both BM and mPB source of cells, while OTL-103-4 used the cryopreserved formulation and mPB source. The Early access programme (EAP) used fresh formulation and mPB cell source.

The progression of the clinical programme is described thereafter:



2.5.5.1. Dose response study(ies)

No formal dose finding studies were conducted.

The minimum dose of 2×10^6 cells/kg was selected based on experience as a recognised threshold for successful engraftment. Similarly, the maximum dose was 30×10^6 CD34+ cells per kg based on observed improved yields in cell harvesting and/or CD34+ cell mobilisation. In the clinical programme, doses ranging 7 to 30.9×10^6 CD34+ cells/kg have been administered as single dose. The actual dose administered in study OTL-103-4 was in the range 23.7-30.9 $\times10^6$ CD34+ cells/kg.

2.5.5.2. Main studies

Title of Study: OTL-103-4: A single arm, open label clinical study of hematopoietic stem cell gene therapy with cryopreserved autologous CD34+ cells transduced with lentiviral vector encoding WAS cDNA in participants with Wiskott-Aldrich Syndrome (WAS).

Methods

This is a phase 3, open label, single arm study.

The study start date was 21-Jan-2019 (1st patient enrolled) and the last patient was treated on 26-Sept-2022. The data cut-off for the initial analysis was 04-Dec-2023; upon request, an updated analysis with DCO 17JAN2025 has been provided. The study is planned for 5 years follow-up and is ongoing. After completion of the 5-year follow up, participants will be monitored for up to 15 years after treatment with Telethon003.

Study Participants

The study was planned to include 10 subjects.

Main inclusion criteria:

1. Diagnosis of WAS defined by genetic mutation and at least one of the following criteria:

a) Severe WAS mutation, defined by literature data, database information and prediction studies (genotype/phenotype studies)

b) Absent Wiskott-Aldrich Syndrome protein (WASP) expression, assessed by flow cytometry

c) Severe clinical score (Zhu clinical score ≥3)

and 2. No human leukocyte antigen (HLA) identical related donor available for hematopoietic stem cell transplantation (HSCT)

Main exclusion criteria:

1. End-organ dysfunction, severe active infection not responsive to treatment or other severe disease or clinical condition which, in the judgment of the Investigator, would make the patient inappropriate for entry into this study
2. Malignant neoplasia (except local skin cancer) or a documented history of hereditary cancer syndrome. Patient with a prior successfully treated malignancy and a sufficient follow-up to exclude recurrence (based on oncologist opinion) could be included after discussion and approval by the Medical Monitor
3. Myelodysplasia, cytogenetic alterations characteristic of myelodysplastic syndrome and acute myeloid leukemia, or other serious hematological disorders
4. Prior allogeneic HSCT, with evidence of residual cells of donor origin
5. Documented human immunodeficiency virus (HIV) infection (positive HIV RNA and/or anti-p24 antibodies)
6. Previous GT

Treatments

Each participant received the investigational medicinal product Telethon003, on a single administration, via IV infusion. The recommended DP dose post-thaw was 3-30×10^6 CD34+ cells/kg. The actual dose was 27.56 (range 23.7-30.9) ×10^6 CD34+ cells/kg. The formulation was cryopreserved Telethon003 and the cellular source was mobilized peripheral blood (mPB).

Table 3. Summary of exposure to telethon003 (intent-to-trat population)

	Telethon003 (N=10) Median (min–max)
Total volume infused (mL)	34.64 (25.1–71.2)
Total nucleated cells (10^6)	330.9 (223–612)
Dose of nucleated cells (10^6/kg)	28.53 (24.1–31.6)
Number of CD34+ cells/kg (10^6/kg)	27.56 (23.7–30.9)
Number of CFU-GM (/10^6 cells)	55,350.0, (0–124,000)[c]
Transduction efficiency (%)	
≤80, n (%)	2 (20.0)
>80, n (%)	8 (80.0)
Vector copy number (VCN/cell)	2.40 (1.4– 3.8)
Percentage of CD34+ cells[a]	98.54 (96.5–100.0)
Effective dose of CD34+ cells (10^6/kg)[b]	22.08 (19.0–24.0)

CFU-GM=colony forming units–granulocyte/macrophage; max=maximum; min=minimum; VCN=vector copy number

a. Percentage of CD34+ cells was calculated as (dose of infused CD34+ cells/kg)/dose of nucleated cells/kg ×100
b. Effective dose was calculated as (dose of infused CD34+/kg)×transduction efficiency
c. The minimum value of "0" was reported erroneously for Participant OTL103-04-11-01 at data cut-off. This information has been provided by the clinical site

All participants had stem cells collected from peripheral blood only.

Participants received the following treatments before Telethon003:

• G-CSF (total dose of 10 to 12.5 µg/kg, in two divided doses) in order to increase mobilization of CD34+ cells from the BM into PB

• On the 4th and 5th days of G-CSF administration, an additional mobilizing agent, plerixafor s.c. once daily at a dose of 0.24 mg/kg,

• Rituximab IV on Day -22 (±1 day), prior to the conditioning regimen, in a single dose of 375 mg/m2

• A reduced intensive conditioning (RIC) regimen:

- Fludarabine (IV): two single doses of 30 mg/m2/day, on Day -4 and Day -3, respectively
- Busulfan (IV) body weight-based and AUC-targeted dose (dose range: 0.8 to 1.2 mg/kg/dose). Eight doses were to be administered every 6 hours from Day -4 to Day -2. If the target AUC was not achieved with eight doses, additional doses were administered every 6 hours, based on AUC measurements. The cumulative target AUC was 48,000 ng/mL·h ±10%. Dosing was to be stopped if the target AUC was reached before the eighth dose. Busulfan dosing was to stop at least 24 hours before the infusion of Telethon003

Concomitant therapy was allowed and included anti-infective drugs, IgRT, RBC and platelet transfusions, granulocyte colony-stimulating factor (G-CSF).

Objectives

The primary objective was to evaluate the clinical efficacy of Telethon003 cryopreserved at 12 months for bleeding events and from 6 to 18 months for severe infections.

Outcomes/endpoints

Primary endpoints:

1. Annualized rate of severe infections from 6 to 18 months after Telethon003 compared with 1 year prior to Telethon003

2. Annualized rate of moderate and severe bleeding episodes up to 1 year after Telethon003 compared with 1 year prior to Telethon003

Secondary efficacy endpoints

Secondary Objective	Evaluation criteria (endpoint)
To evaluate the overall survival at 12, 24, and 36 months	Evaluation of overall survival
To evaluate engraftment at 6 months	1. Hematological reconstitution at 60 days post-treatment (absolute neutrophil count [ANC] >500 cells/ L). Engraftment failure is defined as failure to reach an ANC >500 cells/ L, with no evidence of BM recovery (i.e., hypocellular marrow) on D60 2. Vector copy number (VCN)/cell of >=0.1 in PB-derived CD3+ cells at 6 months

	3. WAS protein expression at 6 months compared with pre-treatment levels in lymphocytes and platelets
To evaluate the biological efficacy of the cryopreserved formulation of Telethon003 at 12 months, 2 years, and 3 years	1. Vector copy number/cell of 0.1 in PB-derived CD3+ cells 2. Wiskott-Aldrich Syndrome protein expression compared with pre-treatment levels in lymphocytes and platelets
To evaluate the clinical efficacy of the cryopreserved formulation of Telethon003 at 2 and 3 years	1. Annualized rate of severe infections from 6 months after Telethon003 compared with 1 year prior to Telethon003 2. Annualized rate of moderate and severe bleeding episodes from Day 1 after Telethon003 3. Platelet count compared with baseline 4. Mean platelet volume (MPV) compared with baseline 5. Eczema severity compared with baseline 6. Presence of autoimmunity phenomena compared with baseline 7. Annualized rate of hospitalization due to infections or bleeding episodes from 6 months after Telethon003 compared with 1 year prior to Telethon003
To evaluate the sustained engraftment at 2 and 3 years	1. 10% lentiviral vector (LVV)-positive clonogenic progenitors in BM 2. Vector copy number/cell of >=0.04 in BM-derived CD34+ cells (multilineage engraftment)
To evaluate the immunological function after treatment with Telethon003 up to 3 years	1. T-cell function (proliferation to stimuli) at 2 and 3 years after Telethon003 2. Response to vaccination up to 3 years after Telethon003, only if performed
To evaluate the effect of Telethon003 on health-related quality of life at 2, and 3 years	Change from baseline in Pediatric Quality of Life Inventory (PedsQL) Generic Core Scale or Infant Scale scores (as appropriate for age)

All analyses were based on descriptive statistics; no formal hypothesis testing was performed. The rate of events was estimated pre- and post-treatment as the number of events divided by the total person-years of follow-up. The efficacy population is the ITT set (treated with Telethon-003 and with data available).

Sample size

Due to the rare nature of this indication, the sample size for this study has been determined based on practical considerations and feasibility. It is not based on statistical considerations. It was planned to treat up to 10 patients, in an estimated 5-year period. All patients were male.

Randomisation and Blinding (masking)

Not applicable.

- **Statistical methods**

Descriptive analysis was performed. No statistical considerations were applied due to be very limited number of patients.

Results

All subjects (n=10) enrolled, underwent PBSC mobilisation, received rituximab and reduced conditioning treatment with busulfan and fludarabine, and received Telethon003 GT treatment.

Table 4. Summary of participant disposition (enrolled set)

	Telethon003 N (%)
Number of participants enrolled	10
Number of participants mobilized	10
Number (%) of participants treated	10 (100)
Number (%) of participants completed 1 year[a, b]	10 (100)
Number (%) of participants completed 2 years[a, b]	10 (100)
Number (%) of participants completed 3 years[a, b]	8 (80.0)
Number (%) of participants completed 4 years[a, b]	6 (60.0)
Number (%) of participants completed 5 years[a, b]	5 (50.0)
Number (%) of participants ongoing[a]	6 (60.0)

a. The denominator for percentage is number of participants treated (Intent-to-treat set)
b. A participant is considered to have completed Year x if their duration of follow-up ≥x
Source: Table 14.1.1.1

Conduct of the study

The Interim CSR for OTL-103-4 study dated 19-Sep-2024, with a data cut-off date for the interim analysis of 4-Dec-2023 was provided. Updated data (up to 17 Jan 25) were provided upon CAT/CHMP request.

The initial protocol (version 1.0) was dated 19-Oct-2018. Several amendments (up to version 6.0, dated 25-Jul-2023 have been performed. Main changes included update of primary endpoints (rate of severe infections and moderate and severe bleeding episodes became primary and engraftment secondary), increased sample size from 6 to 10 patients, and increased duration of follow-up to 5 years followed by LTFU.

Baseline data

All participants in the ITT population were male; the majority of participants were White, and the other 4 were Asian, and Black/African American.

The age of participants at the time of GT ranged from 1 year to 9 years (median age 1.984 years). Eight (80.0%) participants were <5 years of age at the time of GT; five of these eight participants were <24 months of age. Nine (90%) were enrolled at SR-TIGET and One (10%) at the Children's Healthcare of Atlanta (USA). Time from WAS diagnosis to informed consent ranged from 0.24 years to 5.08 years (median 0.603 years).

Table 5. Summary of demographic characteristics at screening/baseline or on date of gene therapy (mobilization set)

Demography	Telethon003 (N=10)
Age on date of gene therapy in years, median (range)	1.964 (0.98–8.98)
Age group on date of gene therapy, n (%)	
28 days to <24 months	5 (50.0)
≥24 months to <12 years	5 (50.0)
Age group (5 year cut-off categories) on date of gene therapy, n (%)	
<5 years	8 (80.0)
≥5 years	2 (20.0)
Sex, n (%) [a]	
Male	10 (100)
Race, n (%) [a]	
Asian	2 (20.0)
Black/African American	2 (20.0)
White	6 (60.0)

Details of WAS gene mutations, baseline WASP expression in leukocytes and platelets, and WAS disease severity in the ITT population are presented in Table 6 below.

Nine (90%) participants had a severe WAS gene mutation, 6/10 (60%) had absent WAS protein expression, 9/10 (90%) had a severity score >=3 (the other subject with Zhu 2.0 had a severe gene mutation).

Table 6. Wiskott-Aldrich Syndrome gene mutations, baseline WASP expression and WAS severity by participant

Participant number	Mutation	Mutation type (Severity of mutation)	% Total lymphocytes expressing endogenous WASP at baseline[a]	Severity of WAS disease (Zhu score)
OTL103-04-11-01	c.389delA p.ASP130ALAFS*131	Deletion (severe mutation)	Not done[b]	3.0
WASCRY01	c.160_165 del p.TYR54_LEU55del	Deletion (severe mutation)	0.60 (absent)	5.0A
WASCRY02	c.892_893del GG p.GLY298TRPFS*37	Deletion (severe mutation)	22.70 (present)	2.0
WASCRY03	c.631 C>T, p.ARG211	Nonsense (severe mutation)	3.90 (absent)	5.0A
WASCRY04	c.1384_1385del AG p.S461LFS*32	Deletion (severe mutation)	1.60 (absent)	5.0A
WASCRY05	c.256C>T p.ARG86CYS	Missense (severe mutation)	6.40% (reduced)	3.0
WASCRY06	c.1058_1061del CACC p.PRO353GLNFSTER91	Deletion (severity not known)	0.80 (absent)	3.0
WASCRY07	c.1097DELG p.GLY366ALAFS*79	Deletion (severe mutation)	9.10 (reduced)	3.0
WASCRY08	c.436DEL p.GLN146LYSFS*115	Deletion (severe mutation)	2.60 (absent)	3.0
WASCRY09	c.37C>T p.ARG13*	Nonsense (severe mutation)	3.50 (absent)	3.0

WAS=Wiskott-Aldrich syndrome; WASP=Wiskott-Aldrich syndrome protein

a. Measured by flow cytometric analysis

b. WASP expression at baseline in this participant was measured in B-cells, monocytes, NK cells and T-cells, resulting absent (<5%) in all these subpopulations

c. For this patient, Zhu score was upgraded to 5A shortly before Telethon003 infusion because of an SAE of "Autoinflammatory flare"

Source: Listing 16.2.4.8 and Listing 16.2.6.3

In terms of clinical manifestations, all (100%) participants had a history of symptoms associated with WAS: 10 (100%) had eczema, 9 had severe infections, 7 (70%) had thrombocytopenia, and 6 (60%) had anaemia.

Numbers analysed

All enrolled subjects (10/10) were included in the efficacy analysis. In the updated analysis with a data cut of (DCO) of 17Jan 2025, the median duration of follow-up is 5.0 years (range: 2.31–5.43 years). 10/10 subjects completed 2 years follow up, with eight participants completing 3 years of follow-up. Six participants had completed the 5-year follow-up visit.

Outcomes and estimation

1. Annualized rate of severe infections 6-18 months post-GT compared to 12 months pre-GT

In the initial analysis, the annualized rate of severe infections decreased following Telethon003 infusion, and no severe infections were reported after more than 2 years of follow-up. The rate of severe infections in the 12 months before Telethon003 infusion was 2.403 (95% CI: 1.5399, 3.5762) per person-year of observation (PYO) and reduced to 0.206 (95% CI: 0.0250, 0.7454) per PYO in the 6-18 months post-GT, and to 0.117 (95% CI: 0.0030, 0.6523) 1-2 years post-GT. The rate of severe infections was 0 from Year 2 through to Year 5 after Telethon003 infusion. All severe infections were CTCAE Grade 3; no Grade 4 infections were reported (Table 23).

In the updated analysis, the rate of severe infections in the 12 months before Telethon003 infusion was 2.403 (95% CI: 1.5399, 3.5762) per person-year of observation (PYO), which reduced to 0.200 (95% CI: 0.0242, 0.7210) per PYO in the 6–18 months post-GT, and to 0.100 (95% CI: 0.0025, 0.5575) 1–2 years post-GT.

Table 7. Summary of number and rate of severe infections events (intent-to-treat population)

Severe infections	Pre-treatment[a] (N=10)	0–6 months[b] (N=10)	6–12 months (N=10)	6–18 months (N=10)	1–2 years (N=10)	2–3 years (N=10)	>3 years (N=8)	>6 months follow-up[c] (N=10)
Person-years of observation	9.99	4.98	5.01	10.02	9.99	8.94	13.54	37.48
Number (%) of participants with severe infection	9 (90.0)	6 (60.0)	1 (10.0)	2 (20.0)	1 (10.0)	0	0	2 (20.0)
Number of severe infections (rate)[d]	24 (2.403)	13 (2.609)	1 (0.200)	2 (0.200)	1 (0.100)	0	0	2 (0.053)
95% confidence interval of the rate	(1.5399, 3.5762)	(1.3891, 4.4613)	(0.0051, 1.1120)	(0.0242, 0.7210)	(0.0025, 0.5575)	(NE, 0.4128)	(NE, 0.2724)	(0.0065, 0.1927)
Grade 3	24 (2.403)	13 (2.609)	1 (0.200)	2 (0.200)	1 (0.100)	0	0	2 (0.053)
Grade 4	0	0	0	0	0	0	0	0
Number of occurrences per participant (%)								
1–5	8 (80.0)	6 (60.0)	1 (10.0)	2 (20.0)	1 (10.0)	0	0	2 (20.0)
6–10	1 (10.0)	0	0	0	0	0	0	0
>10	0	0	0	0	0	0	0	0

NE=not evaluable
a. Events occurring in the 12 months before Telethon003 infusion
b. Events on the day of Telethon003 are not included
c. Events occurring more than 6 months after Telethon003 infusion
d. Rate of events estimated as number of events over person-years of observation
Source: Table 14.2.1.1.3

2. Annualized rate of moderate and severe bleeding events

The combined annualized rate of moderate and severe bleeding events decreased from 0.901 events per PYO in the 12 months before Telethon003 infusion to 0.300 events per PYO in the first 12 months following Telethon003. No severe bleeding events were reported between 6 months and 3 years of follow-up following treatment with Telethon003 (Table 8).

In the updated analysis, the rate of moderate and severe bleeding events decreased from 0.901 events per PYO in the 12 months before Telethon003 infusion to 0.300 events per PYO in the first 12 months and further to 0.200 in the 1-2 years following Telethon003, as shown in the table below:

Table 8. Summary of number and rate of moderate and severe bleeding events (intent-to-treat population)

Bleeding events	Pre-treatment[a] (N=10)	0–6 months[b] (N=10)	0–12 months (N=10)	6–12 months (N=10)	1–2 years (N=10)	2–3 years (N=10)	>3 years (N=8)	Post-treatment[c] (N=10)
Person-years of observation	9.99	4.98	9.99	5.01	9.99	8.94	13.54	42.47
Moderate events								
Number (%) of participants with bleeding event	3 (30.0)	2 (20.0)	2 (20.0)	0	1 (10.0)	0	0	2 (20.0)
Number of bleeding events (rate)[d]	5 (0.501)	2 (0.401)	2 (0.200)	0	2 (0.200)	0	0	4 (0.094)
Severe events								
Number (%) of participants with bleeding event	3 (30.0)	1 (10.0)	1 (10.0)	0	0	0	1 (12.5)	1 (10.0)
Number of bleeding events (rate)[d]	4 (0.401)	1 (0.201)	1 (0.100)	0	0	0	1 (0.074)	2 (0.047)
Moderate and severe events								
Number (%) of participants with bleeding event	6 (60.0)	2 (20.0)	2 (20.0)	0	1 (10.0)	0	1 (12.5)	2 (20.0)
Number of bleeding events (rate)[d]	9 (0.901)	3 (0.602)	3 (0.300)	0	2 (0.200)	0	1 (0.074)	6 (0.141)
95% confidence interval of the rate	0.4121, 1.7109	0.1242, 1.7595	0.0619, 0.8773	NE, 0.7363	0.0242, 0.7230	NE, 0.4128	0.0019, 0.4114	0.0518, 0.3075
Grade 1	0	0	0	0	0	0	0	0
Grade 2	5 (0.501)	2 (0.401)	2 (0.200)	0	2 (0.200)	0	0	4 (0.094)
Grade 3	4 (0.401)	1 (0.201)	1 (0.100)	0	0	0	1 (0.074)	2 (0.047)
Grade 4	0	0	0	0	0	0	0	0
Number of occurrences per participant (%)								
1–5	6 (60.0)	2 (20.0)	2 (20.0)	0	1 (10.0)	0	1 (12.5)	2 (20.0)
6–10	0	0	0	0	0	0	0	0
>10	0	0	0	0	0	0	0	0

NE=not evaluable

a. Events occurring in the 12 months before Telethon003 infusion
b. Events on the day of Telethon003 are not included
c. Events occurring more than 6 months after Telethon003 infusion
d. Rate of events estimated as number of events over person-years of observation

Source: Table 14.2.1.2.3

Secondary objectives

1. Overall survival

All participants in the ITT Population were alive at the time of the data cut-off for this analysis, with follow-up ranging from 2.32–5.43 years in the updated analysis.

2. Haematological reconstitution (ANC >500 cells/L at D60 post-GT)

Nine (90.0%) participants had ANC >500 cells/ L on Day 60; one participant reached hematologic reconstitution by Day 90; all maintained this at all subsequent time points.

3. Engraftment of gene-modified cells in peripheral blood

The main indicator of gene correction was the detection of the WAS LVV sequences in the HSPCs and their progeny. The VCN, (corresponding to the mean number of integrated copies of vector sequences per cell genome), was measured using PCR based methods in DNA samples extracted from BM and PB cell populations at various time points post-treatment.

Engraftment in PB was defined as ≥0.1 VCN/cell in PB CD3+ T lymphocytes (equivalent to 10% gene marked CD3+ cells, assuming a VCN of 1). All 10 participants in the ITT population achieved adequate engraftment with a median time to engraftment of 60.5 days (range: 29-426 days). All evaluable patients maintained engraftment through the DCO.

4. Expression of WAS protein in haematopoietic cells

Immune recovery after WAS GT is dependent on the expression of WASP in the participants' hematopoietic cells. The median percentage of PB cells (platelets, lymphocytes, B-cells, T-cells, NK cells, and monocytes) expressing WASP increased after Telethon003 infusion, peaking between Day 180 and Year 2, and remained above baseline until the data cut-off of this analysis.

Updated analysis:

Table 9. Summary statistics of cells expressing WASP assessed by flow cytometry over time (intent-to-treat population)

% of cells expressing WASP in PB[a]	Baseline	Change from baseline										
	Median (Min, Max) [n]	Day 30 Median (Min, Max) [n]	Day 60 Median (Min, Max) [n]	Day 90 Median (Min, Max) [n]	Day 180 Median (Min, Max) [n]	Year 1 Median (Min, Max) [n]	Year 1.5 Median (Min, Max) [n]	Year 2 Median (Min, Max) [n]	Year 2.5 Median (Min, Max) [n]	Year 3 Median (Min, Max) [n]	Year 4 Median (Min, Max) [n]	Year 5 Median (Min, Max) [n]
% of platelets	14.60 (5.9, 35.8) [8]	59.95 (52.5, 74.3) [6]	55.30 (49.0, 75.7) [7]	50.60 (35.6, 67.8) [8]	61.90 (39.8, 66.5) [6]	62.70 (39.4, 76.8) [5]	73.05 (56.4, 82.6) [6]	70.60 (10.9, 75.7) [7]	66.30 (22.0, 77.8) [3]	61.45 (26.0, 68.2) [6]	52.75 (42.0, 60.1) [4]	55.40 (34.9, 80.3) [5]
% of lymphocytes	3.50 (0.6, 22.7) [10]	10.20 (-19.9, 29.1) [7]	23.25 (-4.0, 48.1) [8]	36.10 (23.1, 55.0) [7]	56.60 (9.2, 65.6) [7]	57.60 (5.2, 81.3) [7]	69.20 (8.8, 84.6) [7]	66.40 (2.6, 94.6) [8]	61.95 (3.9, 67.8) [4]	70.70 (2.4, 77.6) [7]	70.10 (9.8, 82.2) [5]	78.90 (24.9, 84.6) [6]
% of B-cells	2.25 (0.1, 22.1) [10]	6.00 (-5.0, 23.0) [4]	17.55 (3.0, 55.5) [8]	23.05 (8.9, 42.3) [8]	32.40 (4.8, 41.8) [8]	20.45 (-10.2, 82.7) [8]	40.75 (-14.1, 65.0) [8]	28.40 (-14.2, 90.4) [9]	22.40 (-14.5, 44.1) [5]	29.55 (-13.6, 46.6) [6]	22.50 (-9.5, 59.3) [5]	37.30 (-5.1, 69.6) [5]
% of T-cells	1.90 (0.0, 24.8) [10]	-0.15 (-24.7, 0.7) [8]	6.20 (-6.4, 29.1) [9]	18.00 (5.5, 52.1) [8]	62.35 (7.8, 78.1) [8]	73.45 (8.1, 86.2) [8]	79.20 (15.7, 87.7) [8]	79.20 (3.8, 96.8) [9]	76.70 (9.4, 88.0) [5]	74.25 (6.6, 90.3) [6]	73.10 (13.2, 87.2) [5]	85.85 (27.9, 88.8) [6]
% of NK cells	0.95 (0.1, 14.7) [10]	34.85 (-5.1, 45.2) [8]	64.15 (10.0, 77.5) [8]	61.05 (41.2 81.4) [8]	63.80 (29.1, 86.1) [8]	66.20 (5.2 83.0) [8]	70.15 (10.4, 80.9) [8]	66.80 (5.6, 90.5) [9]	50.20 (9.4, 72.2) [5]	67.30 (0.9, 83.3) [7]	71.50 (7.1, 83.4) [5]	74.30 (10.3, 85.0) [6]
% of monocytes	1.25 (0.0, 16.2) [10]	7.20 (-2.7, 40.2) [7]	37.40 (20.5, 56.9) [7]	27.90 (7.3, 45.6) [8]	39.10 (11.5, 58.1) [8]	41.40 (7.2, 64.7) [7]	40.70 (14.8, 58.2) [8]	44.00 (12.1, 67.6) [9]	38.10 (10.4, 53.0) [5]	28.60 (17.2, 51.3) [7]	37.50 (18.6, 39.5) [5]	41.05 (18.2, 57.7) [6]

min=minimum; max=maximum; NK=natural killer; PB=peripheral blood; WASP=Wiskott-Aldrich syndrome protein

a. Expression of WASP was assessed by flow cytometry

If there was more than one value within an assessment window, the earliest available measurement for that visit was used in the summary.

Participants enrolled at the Children's Healthcare of Atlanta site did not have WASP expression assessed in platelets.

Source: Table 14.2.2.4.1

5. Sustained engraftment

Longitudinal sustained engraftment was evaluated by measuring the percentage of LVV-transduced CFU in BM cells (%LVV+ BM-CFU) over time. Engraftment in this study was defined as >=10% LVV+ BM-CFU.

Updated analysis

Table 10. Summary of percentage of lentiviral vector transduced-colony forming units in bone marrow cells (intent-to-treat population)

%LVV* BM-CFU	Day 30	Day 90	Year 1	Year 1.5	Year 2	Year 3	Year 5
n	9	9	8	1	9	7	6
Median (%)	51.0	44.0	49.5	46.0	52.0	36.0	42.9
Minimum, maximum	38, 77	29, 73	17, 83	46, 46	21, 74	10, 72	10.64, 67

BM=bone marrow; CFU=colony forming units; LVV=lentiviral vector
If there was more than one value within an assessment window, the earliest available measurement for that visit was used in the summary.
Source: Table 14.2.2.10.1

6. Clinical efficacy at Year 2 and 3

- By platelet count compared to baseline

Median platelet count was 24.50×10^9/L (range: 11.5- 34.5×10^9/L; n=6) at baseline. Baseline platelet counts could not be included in the analysis for 3 participants who were receiving TPO agonists. Median platelet count increased on treatment starting on D30 through Year 5. At Years 1, 2, 3, 4, and 5 all evaluable participants showed increase in platelet count except for one participant at Year 3. This patient had a Zhu score of 5A at baseline, received a high dose of Telethon003 and a higher than planned dose of Busulfan, which led to serious adverse events. He presented slow and modest increase in platelet count, however he had overall good engraftment parameters. He experienced severe bleeding at Y3 and Y4 and severe infection at Y4, considered autoimmune events. The majority of participants maintained platelet counts above 50×10^9 /L from Day 180 to the data cut-off for this analysis. At baseline, data is available for 4 subjects (40%) and at Year 1 for 4 out of 8 subjects (50%).

The rate of platelet infusions in the ITT population declined steeply after Telethon003 administration. There were 10.29 infusions per PYO in the pre-treatment phase (from the date of screening up to and including the day before the treatment phase), 23.21 infusions per PYO in the on-treatment phase (from the day of PBSC mobilization up to and including Day 1), and 7.43 infusions per PYO in the first 6 months post-Telethon003 (Table 11). No participant required infusions after more than 6 months of follow-up.

Table 11. Summary of number and rate of platelet infusions by treatment phase (intent-to-treat population)

Platelet infusion	Pre-treatment[a] (N=10)	On-treatment[b] (N=10)	0–6 months (N=10)
Person-years of observation	1.36	1.64	4.98
Number (%) of participants with platelet transfusions	5 (50.0)	8 (80.0)	8 (80.0)
Number of platelet transfusions (rate)[c]	14 (10.29)	38 (23.21)	37 (7.43)
95% CI of the rate	(5.63, 17.27)	(16.43, 31.86)	(5.23, 10.24)
Number of occurrences per participant (%)			
1–5	4 (40.0)	7 (70.0)	5 (50.0)
6–10	1 (10.0)	1 (10.0)	3 (30.0)
>10	0	0	0

CI=confidence interval; PYO=person-year of observation
a. From the date of screening up to and including the day before the on-treatment phase
b. From the day of peripheral blood stem cell mobilization up to and including Day 1 (day of Telethon003 infusion)
c. Rate: number of platelet transfusions per PYO
Source: Table 14.2.2.5.6

The cessation of sustained platelet infusions, defined as the onset of a period of 3 months post-Telethon003 without a platelet infusion, was achieved in all eight participants who received platelet infusions post-Telethon003, with a median time to cessation of 33.0 days (range: 10-73 days) after Telethon003 administration.

- By Eczema severity score

Nine participants had eczema at baseline; eczema was transient in five cases and moderate in four participants. Eczema scores improved in 6 and remained stable in 2 participants at Year 1 compared with baseline (no data for 1 participant). At Year 2, in the updated analysis, one out of 9 evaluable participants still had eczema. At Year 3, none of the 7 evaluable participants had eczema. At Year 4 and 5 none of the evaluable participants had eczema.

- By Autoimmune phenomena

Four participants had manifestations of autoimmunity prior to the GT; two were resolved before treatment with waskyra, one resolved within 6 months of treatment, and one had two flares of uveitis at Year 2 and Year 3. In addition, one subject with no autoimmunity before treatment but with severe autoinflammation had two events of autoimmune thrombocytopenia at > Year 3.

- By annualized rate of hospitalization

The rate of hospitalizations was 2.103 per PYO in the 12 months before Telethon003, 0.803 per PYO in the 6-month period following Telethon003. (Table 12).

In the updated analysis, with 2 years follow-up for all subjects, the rate of hospitalization for severe infections and bleeding further decreased to 0 at Year 2 and Year 3. (Updated Table 16).

Table 12. Summary of days and rate of hospitalisation for bleeding and severe infections by treatment phase (intent-to-treat population)

Hospitalization	Pre-treatment[a] (N=10)	0–6 months[b] (N=10)	6–12 months (N=10)	1–2 years (N=10)	2–3 years (N=10)	>3 years (N=8)	>6 months[c] (N=10)
Person-years of observation	9.99	4.98	5.01	9.99	8.94	13.54	37.48
Number (%) of participants with at least one hospitalization	8 (80.0)	4 (40.0)	1 (10.0)	0	0	1 (12.5)	1 (10.0)
Number of hospitalizations (rate)[d]	21 (2.103)	4 (0.803)	1 (0.200)	0	0	1 (0.074)	2 (0.053)
95% CI of the rate	1.3018, 3.2147	0.2187, 2.0554	0.0051, 1.1120	NE, 0.3691	NE, 0.4128	0.0019, 0.4114	0.0065, 0.1927
Days of hospitalization[e]							
Number (%) of participants with at least one day	8 (80.0)	4 (40.0)	1 (10.0)	0	0	1 (12.5)	1 (10.0)
Total days	214	94	9	-	-	3	12
Median (min, max)	10.0 (2, 89)	17.0 (6, 54)	9.0 (9, 9)	-	-	3.0 (3, 3)	12.0 (12, 12)

CI=confidence interval; max=maximum; min=minimum; NE=not evaluable

a. Events occurring in the 12 months before Telethon003
b. Events on the day of Telethon003 are not included
c. Events occurring more than 6 months after Telethon003 infusion
d. Rate of events estimated as number of hospitalizations over person-years of observation
e. Days of hospitalization were counted starting from date of admission to hospital and ended on date of discharge so a period of hospitalization could comprise days counted in multiple phases. A given date was only counted once within each participant, regardless of how many adverse events it was associated with

A hospitalization event was defined as an admission to hospital, where the participant was not already in hospital for another adverse event, and thus was only ever counted in one phase.
Hospitalizations on the day of gene therapy or due to gene therapy are excluded from this table.
Source: Table 14.2.2.9.2

7. Immunological function:

The participants' median in vitro proliferative responses to CD3 stimulation were reduced at baseline compared with the normal range and, from Day 180 onwards, increased when stimulated with CD3i 1 µg/mL and from Year 2 when stimulated with CD3i 0.1 µg/mL up to normal range. A vaccination program was initiated once each participant had discontinued IgRT (at least 3 months after the last Ig supplementation). All vaccinated participants with available data showed a positive serum antibody response to T-cell dependent vaccinal antigens and Pneumococcus vaccine.

8. Quality of life was assessed using the PedsQL scales and family impact module.

In the PedsQL Generic Core Scale, the mean total score in the child self-report increased from 75.06 at baseline to 79.60 by Day 30 post-GT. The score then returned to baseline levels at Day 90 to Day 180, before increasing up to 88.25 at Year 1 and remaining stable through to Year 4.

The PedsQL Family Impact Module was reported by the participant's parent(s). Scaling and scoring were the same as for the PedsQL Generic Score Scale. Mean total score prior to GT was 60.57 at baseline and were stable in the post-treatment phase until Year 2, where the score was 69.86. The mean total score increased to 83.47 in Year 3 and 90.56 in Year 4.

9. Zhu score

In the updated analysis, in all 10 subjects the Zhu score decreased compared to baseline and was 0.5 or 1.0 at the last visit.

Exploratory analyses

- IgRT treatment: 9/10 received IgRT prior to GT, and 10/10 in tge pre-treatment and on-treatment phase up to 6 months post-GT. By the data cut-off date, the cessation of sustained IgRT, defined as the onset of a period of 3 months post-GT without IgRT, occurred in seven participants in the ITT population, with a median time to cessation of 313.0 days (range: 183- 587 day).
- Antimicrobial treatment: Nine of the 10 (90%) participants in the ITT population received antimicrobial medication in the pre-treatment phase and all participants received antimicrobial mediation in the on-, and post treatment phases. At the data cut-off date, sustained (onset of a period of 3 months post-GT without antimicrobial treatment) antimicrobial treatment was discontinued in 7 (70.0%) participants in the ITT population, with a median time to cessation of 377.0 days (range:22.757 days).

2.5.5.3. Summary of main efficacy results

The annualized rate of severe infections decreased from 2.403 (95% CI: 1.5399, 3.5762) per person-year of observation (PYO) to 0.200 (95% CI: 0.0242, 0.7210) per PYO in the 6-18 months post-GT. The number of participants with severe infections decreased from 9/10 in the 12 months pre-GT to 1/10 at Y2 and 0/8 at Y3.

The annualized rate of severe infections further decreased for the 12-24 months post GT to to 0.100 per PYO in the 1-2 years post-Waskyra and to 0 in the 2-3 years post Waskyra.

The annualized rate of moderate and severe bleeding events decreased from 0.901 (0.41- 1.71) events per person year of observation (PYO) 12 months pre-GT to 0.300 in the 12 months following Telethon003 to 0.200 events per PYO in the 1-2 years following Waskyra and 0 events per PYO in the 2-

3 years post-Waskyra. In terms of severe bleeding, the numbers decreased from 3 subjects pre-treatment to 1 subject at Year 1 and none at Year 2.

Nine (90%) participants had haematological reconstitution ANC >500 cells/ L at D60 and 10/10 by D90 and maintained throughout follow-up period.

Engraftment of gene modified cells assessed as the VCN 0.1 VCN/cell in PB CD3+ T lymphocytes (equivalent to 10% gene marked CD3+ cells, assuming a VCN of 1) was reached by D180 in all evaluated subjects and remained stable afterwards.

2.5.5.4. Supportive study(ies)

1. Study TIGET-WAS: A phase I/II clinical trial of hematopoietic stem cell GT for the Wiskott-Aldrich Syndrome

This was a phase 1/2, open label, non-randomized, prospective, single-center study involving a single infusion of autologous CD34+ cells containing HSPCs transduced with LVV encoding the WAS gene.

Key inclusion criteria:

1. Diagnosis of WAS defined by genetic mutation and at least one of the following criteria: severe clinical score (Zhu clinical score >=3), severe WAS mutation, or absent WASP expression

2. No HLA-identical sibling donor and negative search for a matched unrelated donor (10/10) or an adequate unrelated cord blood donor (6/6) within 4 to 6 months or Participants aged >5 years who were not candidates for unrelated allogeneic transplant based on clinical condition

Treatments

The investigational medicinal product was the fresh formulation of Telethon003, a single dose via IV infusion.

The target dose was 5×10^6 to 10×10^6 cells/kg (minimum 2×10^6 cells/kg and maximum 20×10^6 cells/kg) depending on the yield of cells available. The actual dose was 9.56×10^6 cells/kg (range 7.0-16.8).

All eight participants in the ITT population received RIC treatment. The median rituximab dose was 200 mg and the median total fludarabine dose was 60 mg/m2. The median total busulfan dose was 9.5mg/kg and median cumulative AUC was 44,700ng/ml*H.

Primary objectives and endpoints

Objective	Endpoint
To evaluate the safety of the administration of autologous CD34+ cells (Telethon003)	Short-term safety and tolerability of LVV-transduced cell infusion Long-term safety of LVV-transduced cell infusion
To evaluate the long-term engraftment of WASP-expressing transduced cells	Sustained engraftment of genetically corrected HSPCs in PB and/or in BM Expression of vector-derived WASP
To evaluate the efficacy of GT assessed as: a. Improvement of the participant's immune function, specifically T-cell function and antigen-specific responses to vaccinations	Overall survival Improved T cell functions Antigen-specific response to vaccinations

b. Improvement of thrombocytopenia	Improved platelet count and normalization of mean platelet volume (MPV)

Secondary objectives and endpoints

To evaluate the efficacy of GT in improving the participant's clinical conditions, assessed by reduction in frequency of severe infections, bleeding episodes, and reduction of autoimmunity phenomena and eczema.

All analyses were descriptive.

Baseline characteristics

The TIGET-WAS study included 8 subjects. All subjects received treatment with Telethon003 and completed the study (8 years follow-up). Follow-up median duration is 11.1 years (range from 8 to 13.3 years).

Study start date: 20-Apr-2010 (1st subject enrolled)

Study completion date 09-Nov-2023 (refers to completion of 8 years follow-up).

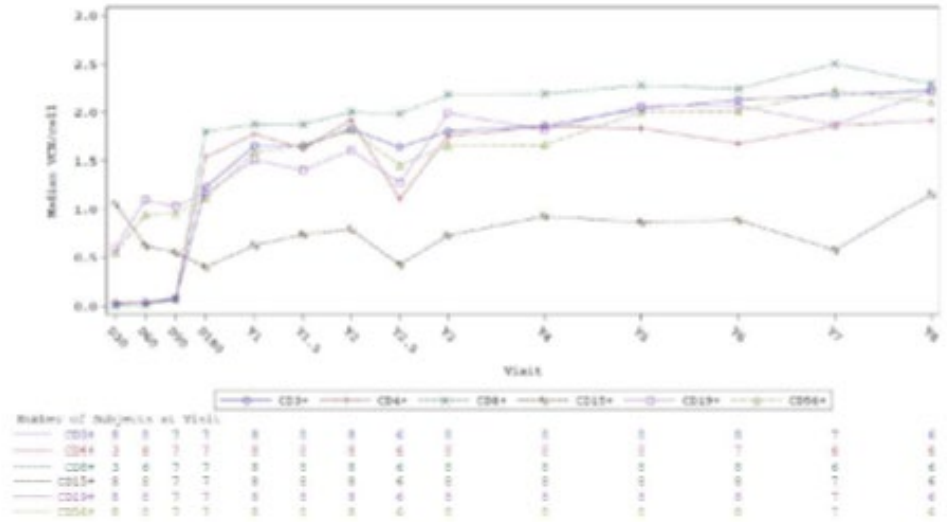
All participants were male; 7/8 [87.5%] participants were White, and one participant was Asian. Median age at the time of GT was 2.2 (range: 1.1-12.4) years. Five (62.5%) participants were aged <5 years, and 4/5 were aged 28 days to <24 months. Median (range) time from WAS diagnosis to the date of informed consent was 0.70 (0.02-1.70) years. Five of eight participants were aged under 18 years at the end of the study and three participants were aged 18 years or over.

Outcomes and estimations

Overall survival: All eight (100%) treated with Telethon003 were alive at the end of follow-up, with a follow-up duration ranging from 8.0 to 13.3 years.

Engraftment of genetically corrected cells, defined as >=0.04 VCN/cell in BM CD34+ cells or >=0.1 VCN/cell in PB CD3+ T-cells, was observed in all (100%) participants and was achieved by a median of 32.5 days after Telethon003 infusion (range: 28–34 days). Engraftment was maintained in all participants at 5 years (n=8) and >=8 years (n=7) after Telethon003 infusion. Sustained multilineage engraftment, defined as 0.04 VCN/cell, was achieved in all evaluated BM (CD3+, CD15+, CD19+, CD56+, and glycophorin A+) and PB (CD3+, CD15+, CD19+, and CD56+) cell subpopulations.

Figure 7. Median values for VCN/cell in peripheral blood cell lineages (intent-to-treat population)



Median percentage of LVV transduced-colony forming units (CFU) in BM cells (chimerism) was 54.40% at the Day 30 visit. The proportion of transduced cell chimerism within the total population remained stable over time.

Table 13. Summary of percentage of lentiviral vector transduced-colony forming units in bone marrow cells (intent-to-treat population)

%LVV* CFU in BM	Day 30 (N=8)	Day 90 (N=7)	Year 1 (N=8)	Year 2 (N=8)	Year 3 (N=6)	Year 5 (N=4)	Year 8 (N=6)	Year 9 (N=1)
Median	54.40	43.50	42.80	41.00	50.30	71.00	66.50	32.00
Min, max	18.0, 77.0	12.5, 81.0	22.0, 85.0	20.7, 86.0	23.5, 91.0	30.5, 100.0	30.0, 100.0	32.0, 32.0

BM=bone marrow; CFU=colony forming units; COVID-19=coronavirus disease 2019; LVV=lentiviral vector; max=maximum; min=minimum

If there was more than one value within an assessment window, the earliest available measurement for that visit was used in the summary.

The 8-year visit for one participant was delayed to 8.76 years post-treatment due to the COVID-19 emergency and the data generated during this visit were therefore windowed to Year 9 for analysis, as described in the Statistical Analysis Plan.

Source: Table 14.2.1.2.6

WASP expression: The median percentage of platelets expressing WASP by flow cytometry was 18.7% at baseline, reached 85.7% by Day 30, approximately 90% at Days 60 and 180, subsequently declined slightly up to Year 3, and stabilized above 60% until the final available timepoint at Year 9. The median percentage of WASP-expressing lymphocytes gradually increased from 3.67% at baseline to 53.30% at Day 180, above 70% at all but one timepoint from 1 year after Telethon003 infusion and 80% as from 3 years post-treatment onwards.

T cell function: Positive T-cell proliferation responses to stimulation with anti-CD3 antibody were observed after Telethon003 infusion.

Response to vaccination: Over the whole follow-up period, seven (87.5%) participants showed a positive serum antibody response. All vaccinated participants exhibited an antibody response to Pneumococcus up to Year 8, a cellular response to tetanus toxoid and to live attenuated measles and rubella vaccine and/or varicella vaccines.

Platelets count: All participants had sustained increased Platelet counts following Telethon003 infusion, although values remained below the normal range.

The rate of platelet infusions decreased from 35.0 per person-year of observation (PYO) pre- treatment to 0.3 per PYO from 6 months after Telethon003. Sustained platelet infusions were discontinued in all participants and median time to cessation (defined as the onset of a 3-month period post Telethon003 without platelet infusions) was 85.5 days after Telethon003 infusion (range: 15–261 days).

Bleeding events: The rate of bleeding events declined from 6 months after Telethon003 infusion, with a combined rate for moderate and severe events of 3.4 events per PYO in the 12 months before Telethon003 infusion, 3.3 in the 6 months post-GT, 0.5 in 6-12 months, and 0.1 from Year 1 onwards (except for Year 3: 0.2).

Severe infections: The rate of severe infections (Grade 3 or 4) decreased from 2.1 per PYO in the 12 months before Telethon003 infusion to 0.1 per PYO from 6 months after Telethon003 infusion.

Table 14. Summary of number and rate of severe infections (intent-to-treat population)

Severe infections	Pre-treatment[a] (N=8)	0–6 months[b] (N=8)	6–12 months (N=8)	1–2 years (N=8)	2–3 years (N=8)	3–8 years (N=8)	>8 years (N=8)	>6 months[c] (N=8)
Person-years of observation	8.00	3.99	4.01	7.99	8.02	39.99	22.38	82.39
Number (%) of participants with severe infection	5 (62.5)	4 (50.0)	0	2 (25.0)	2 (25.0)	2 (25.0)	1 (12.5)	5 (62.5)
Number of severe infections (rate[d])	17 (2.1)	22 (5.5)	0	2 (0.3)	2 (0.2)	4 (0.1)	1 (0.0)	9 (0.1)
95% confidence interval of the rate	1.2, 3.4	3.5, 8.4	NE, 0.9	0.0, 0.9	0.0, 0.9	0.0, 0.3	0.0, 0.2	0.0, 0.2
Grade 3	17 (2.1)	20 (5.0)	0	2 (0.3)	1 (0.1)	4 (0.1)	1 (0.0)	8 (0.1)
Grade 4	0	2 (0.5)	0	0	1 (0.1)	0	0	1 (0.0)
Number of occurrences per participant, n (%)								
1–5	4 (50.0)	2 (25.0)	0	2 (25.0)	2 (25.0)	2 (25.0)	1 (12.5)	5 (62.5)
6–10	1 (12.5)	2 (25.0)	0	0	0	0	0	0
>10	0	0	0	0	0	0	0	0

E=number of events; NE=not evaluable

a. Pre-treatment: events occurring in the 12 months before Telethon003 infusion
b. Events on the day of Telethon003 are not included
c. Events occurring more than 6 months after Telethon003 infusion
d. Rate of events estimated as number of events over person-years of observation

Source: Table 14.2.2.2.4

All participants received IgRT in the pre-treatment phase and in the first 12 months after Telethon003 infusion. Sustained IgRT was discontinued in all participants and median time to cessation (defined as the onset of a 3-month period post Telethon003 without IgRT) was 577.0 days after Telethon003 infusion (range: 307–1843 days).

Sustained antimicrobial treatment was discontinued in all participants and median time to cessation (defined as the onset of a 3-month period post Telethon003 without antimicrobial treatment) was 398.0 days after Telethon003 infusion (range: 138–2534 days).

Auto-immunity and auto-inflammatory manifestations: the rate decreased and stabilized after Telethon003 treatment.

Eczema: Seven participants had eczema (transient to severe) at baseline, and this had resolved in all seven participants (i.e., no eczema) by 6 years after Telethon003 infusion.

Hospitalization: Hospitalizations for events not related to study treatment increased from 3.8 hospitalizations per PYO in the 12 months before GT to 4.3 hospitalizations in the first 6 months after Telethon003, but then decreased to 0.2 hospitalizations per PYO from 6 months post-treatment onwards.

2. EAP Expanded access program (EAP)

The EAP includes the Hospital exemption (HE) and compassionate use program (CUP).

Table 15. Key inclusion criteria

Criterion	Hospital Exemption	Compassionate Use Program
1.	Diagnosis of WAS defined by genetic mutation	Diagnosis of WAS defined by genetic mutation
2.	Severe clinical score (Zhu clinical score ≥3)	Severe disease defined as fulfillment of at least 1 of the following criteria: Severe clinical score (Zhu clinical score ≥3) Absent WASP expression Severe WAS mutation Family history positive for WAS disease with life threatening or fatal clinical events
3.	No HLA identical sibling donor	No HLA identical sibling donor
4.	Negative search for a MUD (10/10) or an adequate unrelated cord blood donor (6/6) within 4 months OR Age >5 years and clinical conditions for which other allogeneic transplantation approaches were not a valid alternative	Negative search for a MUD (10/10) or an adequate unrelated cord blood donor (6/6) within 3 months • This would be reduced to 2 months in the case of a compromised or unstable patient in order to reduce the risk of potential occurrences of severe infections/bleeding events during the waiting time, upon indication from the San Raffaele responsible physician, and following discussion with the Medical Monitor OR Patients >5 years of age who were not suitable for unrelated allogeneic transplant, based on their clinical condition
5.	Parental/guardian/patient signed informed consent	Parental/guardian/patient signed informed consent

HLA=human leukocyte antigen; MUD=matched unrelated donor; WAS=Wiskott-Aldrich syndrome; WASP=Wiskott-Aldrich syndrome protein

Treatments

For the nine patients who received Telethon003 (ITT population), the median number of CD34+ cells infused was 15.50×10^6 cells/kg (range 11.2 - 26.4). Median transduction efficiency was high at 88.0%, and the median VCN per cell was 1.80 copies/cell (range 0.9 – 4.0). No patient required infusion of the stem cell backup. The CD34+ cell source was mPB for all patients.

The reduced conditioning regimen administered was the same as for the main study and TIGET-WAS study.

Objectives and study assessments

The objective of the EAP was to provide treatment for patients affected by WAS with high unmet medical need. Study assessments included clinical evaluation, Zhu score, WAS mutation and WASP expression, diagnostic imaging, routine laboratory determinations, BM evaluation, microbiological and immunological evaluation, presence of gene-corrected cells, platelet function, evaluation of autoimmunity. Assessments were scheduled at regular intervals up to Year 8 post-GT, after which the subjects were transferred to a LTFU study.

Nine patients received the RIC and Telethon003 infusion and were included in the Intent-to-Treat (ITT) population. One patient died approximately 4.5 months after Telethon003 infusion (see below).

Study start date: 14-Dec-2015 (HE), 29-Nov-2016 (CUP) (1st subject enrolled)

Study completion date 17-Oct-2023 (HE) (refers to completion of 8 years follow-up).

Date of last completed visit: 10-Oct-2023

Data cut-off date: 24-Oct-2023

By the cut-off date, 8/9 patients had completed the Year 3 visit, with 7/9 patients having also completed the Year 5 visit. Median duration of follow-up in surviving patients was 5.948 (range: 4.73-7.44) years. Excluding the patient who died, all patients were followed for at least 4 years post-GT.

Baseline data

All patients were male; the majority were white and the other were Asian and American Indian. Median age at the time of GT was 3.8 (1.4-35.1) years: 5/9 (55.6%) were aged <5 years, 2/5 were aged 28 days to <24 months, 2/9 were aged 28 and 35 years.

Outcome and estimation

Overall survival: Eight of nine patients in the ITT population were alive at the time of data cut-off for this final analysis. One participant died due to a SAE that was considered unrelated to Telethon003 by the treating physician.

Engraftment of genetically corrected cells, defined as >=0.04 VCN/cell in BM CD34+ cells or >=0.1 VCN/cell in PB CD3+ T-cells, was observed in all nine patients and was achieved at a median of 32.0 days after Telethon003 infusion (range: 26–35 days). Engraftment was stable and maintained in all evaluable patients at all timepoints.

Median percentage of LVV transduced-CFU in BM cells (chimerism) was 43.5% at the Day 30 visit. The proportion of transduced cell chimerism within the total population remained stable over time.

WASP expression: The median percentage of PB platelets expressing WASP by flow cytometry was 13.0% at baseline and reached 54.5% by Day 30 (n=7); it further increased and remained above 70% from Year 2 until the final available timepoint at Year 7. The median percentage of PB lymphocytes expressing WASP was 28.4% at baseline and increased to values exceeding 70% as from Year 2.

Responses to vaccination: All eight surviving patients received vaccinations within the first 3 years after Telethon003 infusion (after IgRT had been discontinued) and exhibited a positive serum antibody response to vaccination, indicating the restoration of B-cell function.

Platelet counts increased in all patients following Telethon003 infusion, and this was sustained over time.

The rate of platelet infusions decreased from 8.4 per PYO pre-treatment to 0.2 per PYO from 6 months after Telethon003. Sustained platelet infusions were discontinued in all patients and the median time to cessation (defined as the onset of a 3-month period post-Telethon003 without platelet infusions) was 20.0 days after Telethon003 infusion (range: 9–68 days).

The rate of bleeding events declined from 6 months after Telethon003 infusion, with a combined rate for moderate and severe events of 2.0 events per PYO in the 12 months before GT, 1.1 events per PYO in the first 6 months after Telethon003, and 0.1 events per PYO in the >6 months follow-up.

The rate of severe infections (Grade 3 or 4) decreased from 1.4 infections per PYO in the 12 months before GT to 0.1 per PYO from 6 months afterward.

All patients received prophylactic and curative antimicrobial medication in the pre-, on- and post-treatment phases. Sustained antimicrobial therapy was discontinued in all patients within a median time of 422.0 days (range: 207–1163 days) after Telethon003 infusion.

Seven patients had eczema (transient to severe) at baseline, and this had resolved in all seven patients (i.e., no eczema) by 4 years after Telethon003 infusion.

The hospitalization rate for events not related to the study treatment increased from 1.4 hospitalizations per PYO in the 12 months before Telethon003, to 2.1 hospitalizations per PYO in the first 6 months after Telethon003 but then decreased to 0.1 hospitalizations per PYO from 6 months post-treatment onwards.

3. Analysis across trials (Integrated efficacy analysis)

Efficacy data from the TIGET-WAS and OTL-103-4 studies and the EAP have been integrated in order to assess the clinical efficacy of Telethon003 in a combined analysis. At the latest data cut off and following updated analysis provided upon CAT/CHMP request, the 26 surviving participants had a median follow-up of 6.816 years (range 2.31 to 13.26 years). The median age at the time of Telethon003 treatment was 2.2 years in TIGET-WAS, 1.984 years in OTL-103-4, and 3.841 years in the EAP. The clinically relevant biological and clinical manifestations were collected in the same way in the two studies and the EAP, which allowed these data to be integrated.

In the combined analysis, 27 patients have been treated with Telethon003; one patient in the EAP died at 4.5 months after GT.

In the updated analysis with data cut off 17 Jan 2025, for the integrated analysis, data is available for 26/27 patients for 2 years, 20/27 for 5 years, 8/27 for 8 years, 5/27 for 10 years and 1/27 for 13 years.

Table 16. Summary of participant disposition (Efficacy population)

	Telethon003
Number (%) of participants in the Integrated Summary of Efficacy population	27 (100)
Number (%) of participants completed 6 months	26 (96.3)
Number (%) of participants completed 1 year	26 (96.3)
Number (%) of participants completed 1.5 years	26 (96.3)
Number (%) of participants completed 2 years	26 (96.3)
Number (%) of participants completed 2.5 years	25 (92.6)
Number (%) of participants completed 3 years	24 (88.9)
Number (%) of participants completed 4 years	22 (81.5)
Number (%) of participants completed 5 years	20 (74.1)
Number (%) of participants completed 8 years	8 (29.6)
Number (%) of participants completed 10 years	5 (18.5)
Number (%) of participants completed 13 years	1 (3.7)
Number (%) of participants ongoing	6 (22.2)
Number (%) of participants completed the Study [a]	4 (14.8)
Number (%) of participants withdrawing from the study/program [b]	17 (63.0)
Study withdrawal/completion reason n (%)	
Death	1 (3.7)
Study/Program closed	16 (59.3)

Source: Table 2.7.3.1.1.1

a: Subjects from the OTL-103-4 study considered to have completed the study if they have completed up to and including the 5-year follow-up visit post gene therapy and have all Year 5 data in the clinical data base at data cut of 17-JAN-2025.

b: Subjects from the Pivotal Study TIGET-WAS and the Expanded Access Program (HE/CUP) were considered as withdrawals at the date of closure of the respective study or program.

For the 26 participants alive at the data cut-off, the median duration of follow-up was 5.72 years (range 1.19-13.26).

Treatments

In Study TIGET-WAS, eight participants were treated with the fresh formulation of Telethon003. The cellular source material was harvested from the participant's bone marrow (BM) via BM aspiration or from mPB via leukapheresis after PBSC mobilization.

In Study OTL-103-4, 10 patients were treated with the cryopreserved formulation, while mPB was selected as the only source material for future manufacturing of the cryopreserved formulation of Telethon003.

In the EAP program, 9 patients were treated with the cryopreserved formulation and mBP source material for Telethon003.

Objectives

The primary efficacy endpoints for this integrated summary are as follows

- Overall survival

- Rate of moderate and severe bleeding events in the first 12 months post-treatment

- Rate of severe infections from 6 to 18 months post-treatment

Secondary efficacy endpoints are:

- Engraftment of genetically corrected hematopoietic stem cells in PB and BM over time

- WASP expression over time: Percentage of lymphocytes, T-cells, B-cells, NK cells, monocytes, platelets expressing WASP

- T-cell function over time: Dose response of proliferation to anti-CD3i antibody at 0.01, 0.1 and 1 µg/mL expressed as both stimulation index (SI) and counts per minute (cpm)

- Platelet count

- Autoimmunity phenomena

- Eczema score over time: Categorized as either none, transient, moderate, severe

Baseline data (updated analysis)

All participants were male and the majority (20 [74.1%] participants) were White. The age of participants at the time of GT ranged from 1−35.1 years, with 11 (40.7%) participants aged 28 days to <24 months, 11 (40.7%) participants aged ≥24 months to 11 years, and five (18.5%) participants aged >11 years. An important proportion of participants (33.3%) were aged ≥5 years at the time of treatment.

In terms of characteristics of disease, 13/27 (48.1%) had a Zhu score of 3.0 and 4/27 of 4 and 8/27 of 5A the Zhu score was missing for one subject). The WAS protein expression was not absent (present, reduced, or revertant) in 11 subjects and 'absent' in 15 (missing in one subject). The WAS mutation was Class 1 in 5 subjects and Class 2 in 22 subjects.

All participants had thrombocytopenia at baseline, and in the 12 months pre-GT treatment 19/27 had severe infections and bleeding events, 23/27 had eczema, 11/27 had autoimmune manifestations and 9/27 had auto-inflammation.

Outcomes

Overall survival: Twenty-six participants (96%) (95% CI: 82, 99%) in the Efficacy population were alive at the time of the integrated analysis (up to 13 years for 1 subject).
In the updated analysis, the rate of severe infections decreased dramatically from 2.001 (95% CI: 1.5033-2.6110) infections per PYO in the 12 months period before GT to 0.154 (95% CI: 0.0418-0.3931) infections per PYO in the 6–18 month and 1-2 years post-Telethon003) and to 0.046 at Y5. The number of participants with severe infections decreased from 19/27 in the 12 months pre-GT to 3/26 at Y2 and 1/20 at Y5.

Table 17. Summary of number and rate of severe infections (Efficacy population)

Severe Infections	Pre-Treatment (N=27)	0-6 Months (N=27)	6-12 Months (N=26)	6-18 Months (N=26)	1-2 Years (N=26)	2-3 Years (N=26)	3-5 Years (N=24)	>6 Months Follow-up (Up to 54 Years) (N=26)	>5 Years Follow-up (N=20)	>6 Months Follow-up (N=26)
Person-years of observation	26.99	13.33	13.03	26.08	25.98	24.97	43.75	107.73	56.61	164.26
Number (%) of participants with severe infection	19 (70.4)	16 (59.3)	2 (7.7)	4 (15.4)	4 (15.4)	3 (11.5)	1 (4.2)	8 (30.8)	1 (5.0)	9 (34.6)
Number of severe infections (rate)	54 (2.001)	42 (3.152)	2 (0.154)	4 (0.154)	4 (0.154)	3 (0.120)	2 (0.046)	11 (0.102)	3 (0.053)	14 (0.085)
95% confidence interval of the rate	1.5033, - 2.6110	2.2716–4.2605	0.0186–0.5546	0.0418–0.3931	0.0419–0.3942	0.0248–0.3511	0.0055–0.1651	0.0510–0.1827	0.0109–0.1549	0.0466–0.1430
Grade 3 (%)	54 (2.001)	40 (3.002)	2 (0.154)	4 (0.154)	4 (0.154)	2 (0.080)	2 (0.046)	10 (0.093)	3 (0.053)	13 (0.079)
Grade 4 (%)	0	2 (0.150)	0	0	0	1 (0.040)	0	1 (0.009)	0	1 (0.006)

The rate of moderate and severe bleeding events decreased from 2.001 (95% CI: 1.5033-2.6110) events per PYO in the 12 months before GT to 0.797 (95% CI: 0.4933-1.2182) events per PYO in the 12 months, 0.154 in the 1-2 years following Telethon003 , 0,160 events in the 2-3 years and 0.018 (95% CI: 0.0004-0.0984) events per PYO in the >5-year period. The number of patients with moderate and severe bleeding decreased from 19/27 in the 12 months pre-GT to 10/26 in the 12 months post-GT, to 3/26 at Y2 and to 3/20 at Y5.

Table 18. Summary of moderate and severe bleeding events (Efficacy population)

Bleeding Events	Pre-Treatment (N=27)	0-6 Months (N=27)	6-12 Months (N=27)	6-12 Months (N=26)	1-2 Years (N=26)	2-3 Years (N=26)	3-5 Years (N=24)	Post-Treatment (Up to 5 Years) (N=27)	>5 Years Follow-up (N=20)	Post-Treatment (N=27)
Moderate + Severe Events										
Person-years of observation	26.99	13.33	26.35	13.03	25.98	24.97	43.75	121.05	56.61	177.59
Number (%) of participants with bleeding event	19 (70.4)	9 (33.3)	10 (37.0)	2 (7.7)	3 (11.5)	2 (7.7)	3 (12.5)	11 (40.7)	1 (5.0)	11 (40.7)
Number of bleeding events (Rate)	54 (2.001)	19 (1.426)	21 (0.797)	2 (0.154)	4 (0.154)	4 (0.160)	3 (0.069)	32 (0.264)	1 (0.018)	33 (0.186)
95% confidence interval of the rate	1.5033–2.6110	0.8585–2.2267	0.4933–1.2182	0.0186–0.5546	0.0419–0.3942	(0.0436, 0.4102)	(0.0141, 0.2004)	(0.1808, 0.3732)	(0.0004, 0.0984)	(0.1279, 0.2610)
Grade 2	30 (1.112)	17 (1.276)	19 (0.721)	2 (0.154)	4 (0.154)	4 (0.160)	2 (0.046)	29 (0.240)	0	29 (0.163)
Grade 3	23 (0.852)	2 (0.150)	2 (0.076)	0	0	0	1 (0.023)	3 (0.025)	1 (0.018)	4 (0.023)
Grade 4	1 (0.037)	0	0	0	0	0	0	0	0	0
Number of occurrences per participant (%)										
1–5	17 (63.0)	9 (33.3)	10 (37.0)	2 (7.7)	3 (11.5)	2 (7.7)	3 (12.5)	10 (37.0)	1 (5.0)	10 (37.0)
6–10	2 (7.4)	0	0	0	0	0	0	1 (3.7)	0	1 (3.7)
>10	0	0	0	0	0	0	0	0	0	0

No engraftment failure [defined as ANC <=500 cells/µL and no evidence of BM recovery (i.e., hypocellular BM) at Day 60] was recorded in any participant.

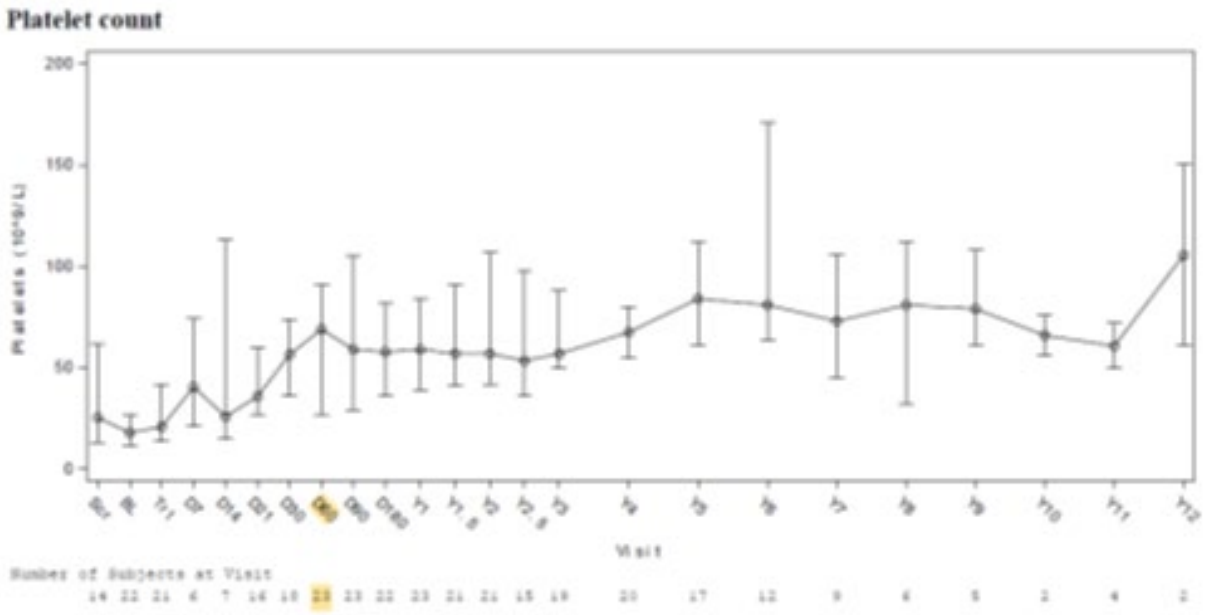
Twenty-five of 27 (92.6%) participants had an ANC >500 cells/µL at 60 days post-Telethon003. At Day 90, all (100%) participants had ANC >500 cells/µL and maintained throughout the follow-up.

All participants in the efficacy population achieved adequate engraftment of BM CD34+ and/or PB CD3+ cells, defined as either >=0.04 VCN/cell in BM CD34+ cells or >=0.1 VCN/cell in BM CD3+ cells by 35 days after infusion of Telethon 003. Median time to engraftment was 32.0 days (range: 26-35). Median time to engraftment of PB CD3+ T cells alone was 91.0 days (range: 29-426). Overall, a durable and stable engraftment of gene corrected cells was observed from 1 month after the single administration of Waskyra and throughout post-treatment follow-up, in all evaluated participants (n=26) with a follow-up period ranging between 2 and 9 years after gene therapy (GT). All participants showed a marked increase in WASP expression in platelets and lymphocytes, and an increase in platelet count and improved T-cell functionality.

The proportion of genetically corrected cells in BM-derived clonogenic cell progenitors in all participants evaluable at Years 1 and 2 was around 50% LVV+ BM-CFU and the median values of LVV+ BM-CFU reported were very similar over the 2-year time period.

All participants with WAS had thrombocytopenia at baseline, and 23 out of 27 were receiving platelet infusions at the time of treatment. Platelet count and MPV increased relative to baseline following Telethon003 infusion until Day 60 to Day 90, whereupon both parameters plateaued and remained relatively stable until the data cut-off.

Figure 8. Median (95% confidence interval) values of mean platelet count and platelet volume over time (Efficacy population)



The combined annualized rate of platelet infusions decreased from 10.824 (95% CI: 8.7873-13.1908) infusions per PYO in the pre-treatment phase to 1.689 (95% CI: 1.0584-2.5569) infusions per PYO in the 6-12-month period, 0.077 (0.0093−0.2781) at Year 2 and 0.080 (0.0097,0.2893) after Telethon003 infusion. The overall rate was 0.170 (95% CI: 0.1133−0.2464) infusions per PYO in the entire >6 months post-treatment.

Based on the Investigators' review, 2 participants in Study TIGET-WAS, 4 participants in Study OTL-103-4, and 4 patients in the EAP had clinical manifestations of autoimmunity prior to GT. Transient clinical manifestations of autoimmunity post-GT included: 4 events of immune thrombocytopenia in 4 (14.8%) participants and one event of autoimmune neutropenia in one (3.7%) participant during the first 6 months after Telethon003 infusion, as well as one event of uveitis in one (3.7%) participant in the 1–2 year period, one event of Grave's disease in one (4.5%) participant in the 3–8 year period and one event of Henoch-Schonlein purpura in one (12.5%) participant in the >8-year period.

Eczema: Twenty-three out of 27 participants had eczema at baseline; eczema was transient in 14 participants, moderate in six participants, and severe in three participants. No participant had a score of either "moderate" or "severe" at any timepoint from Year 1 onwards.

The annualized rate of hospitalizations for bleeding and severe infections decreased from 1.890 hospitalizations per PYO in the 12 months before GT to 0.084 hospitalizations per PYO in the >6-month period post-Telethon003 and decreased further to 0.088 hospitalizations per PYO in the >5-year period.

Analysis by age (< 5 years and > 5 years)

The integrated analysis allowed for higher sample size for subgroup analysis by age (< 5 years and > 5 years) and severity of disease (Zhu score and WASP expression at baseline). At the time of analysis, 8/9 participants in the ≥5 years age group and all 18 participants in the <5 years age group were alive.

In participants <5 years, the rate of infections decreased from 2.502 (95% CI: 1.83; 3.34) infections per PYO in the 12 months period before GT to 0.222 (0.0269, 0.8011) in the 6-12 months, 0.242 (0.0659, 0.6193) in the 1-2 years and 0.143 (0.0173, 0.5150) in the 2-3 years period after treatment. The overall rate was 0.129 ((95% CI: 0.0689, 0.2214) infections per PYO >6-month follow-up.

In participants ≥5 years, the rate of infections was 1.0 (95% CI: 0.46; 1.90) infection per PYO in the 12 months period before GT decreased 0 in the 6-12 months, 1-2 years periods up to 0.125 (0.0173, 0.5150) in the 2-3 years period after treatment. The overall rate was to 0.019 (95% CI: 0.0005, 0.1038) in the >6-month follow-up.

2.5.6. Discussion on clinical efficacy

The initially claimed indication was "Treatment of Wiskott-Aldrich Syndrome in patients aged 6 months and older who have a mutation in the WAS gene and for whom no suitable HLA-matched related HSCT donor is available". This indication was further updated upon CAT/CHMP request to further clarify the study population as follows:

"Treatment of patients aged 6 months and older with Wiskott-Aldrich Syndrome (WAS) who have a mutation in the WAS gene for whom haematopoietic stem cell (HSC) transplantation is appropriate and no suitable human leukocyte antigen (HLA)-matched related haematopoietic stem cell donor is available".

The clinical development program consisted of a phase 3 pivotal study (OTL-103-4), a phase 1/2 study (TIGET-WAS) and an expanded use program (EAP) comprised of hospital exemption (HE) and compassionate use program (CUP).

Two formulations, fresh and cryopreserved and two sources of starting material were used in clinical development: study TIGET-WAS used the fresh formulation and both BM and mPB source of cells, while OTL-103-4 used the cryopreserved formulation and mPB source. The EAP used fresh formulation and mPB cell source.

Dose selection

The Telethon003 DP dose range administered to participants in the efficacy population was $7.0–30.9\times10^6$ CD34+ cells per kg. It is noted that the lower bound of the recommended dose range is driven by data from TIGET-WAS (7.0 to 16.8 $\times10^6$ cells/kg with BM cellular source) while the upper bound is driven by data from OTL-103-4 (23.7 to 30.9 $\times10^6$ cells/kg with mPB cellular source). Comparability of formulations has been demonstrated during the drug development and no difference in clinical outcome were noted.

Design and conduct of clinical studies

The clinical studies (TIGET-WAS and OTL-103.4) were open-label single arm studies that have enrolled 18 WAS patients and had clinical efficacy endpoints of annualised rates of severe infections and moderate/severe bleeding episodes.

The EAP also used the 12-month pre-treatment period as comparator for clinical efficacy endpoints of annualised rates of severe infections and moderate/severe bleeding episodes. Nine treated patients were enrolled.

Main study: OTL-103-4

The phase 3 study OTL-103-4 was a single arm, open label, non-randomized study for evaluation of Telethon003 in subjects with WAS disease. The study design has been endorsed in the CHMP protocol assistance and the endpoints were considered relevant as the main manifestations of the WAS disease The study included 10 subjects and was conducted at Ospedale San Raffaele, Milan Italy (9 patients treated) and Children's Healthcare of Atlanta, US (1 patient). The study drug was Telethon003, as a single administration via IV infusion. The study is ongoing for 5 years. Afterwards all participants will be enrolled in the LTFU study with a follow-up of 15 year, in line with the EMA guidance for integrative viral vectors-based gene therapy.

The inclusion is based on diagnosis of WAS defined by genetic mutation and at least one of the following criteria: 1) severe WAS mutation, 2) absent WAS protein expression, 3) severe clinical score (Zhu score) and Patients were eligible if no human leukocyte antigen (HLA) identical related donor available for hematopoietic stem cell transplantation (HSCT).

The recommended dose was 3-30×10^6 CD34+ cells/kg and the actual dose administered in study OTL-103-4 was in the range 23.7-30.9 ×10^6 CD34+ cells/kg,). The formulation used was the cryopreserved formulation and the cell source was mPB, representative of the commercial drug product.

Prior to the leukapheresis, treatment with G-CSF and plerixafor for cell mobilization was performed, followed by pre-conditioning with rituximab (an anti-CD20 monoclonal antibody) and a reduced conditioning myelo-ablative regimen consisting of busulfan and fludarabine.

The primary endpoint was the annualized rate of severe infections 6-18 months post-GT and respectively moderate and severe bleeding events within 12 months post-GT compared to the respective rate of events in the 12-month period pre-GT. The endpoints are relevant as the main manifestations of the WAS disease. The endpoints, descriptive analysis, and comparison to the pre-GT period were considered appropriate in absence of feasible comparator. The endpoints and comparator were agreed in the CHMP protocol assistance.

Several secondary and tertiary objectives were defined and together contribute to a comprehensive assessment of efficacy up to 3 years post-GT. Secondary objectives include Overall survival, engraftment efficiency and sustained response, clinical efficacy parameters (infections, bleeding, eczema, etc.), immunological function, and safety of treatment up to 3 years post GT. Exploratory analysis included safety parameters (RCL, anti-WASP antibodies), abnormal clonal proliferation, quality of life, and platelet activation up to 3 years.

Supportive studies

TIGET-WAS: The study Tiget-WAS was a single arm, open label, non-randomized study for evaluation of Telethon003 in subjects with WAS disease and enrolled 8 pediatric patients. The study was conducted in a single centre at Ospedale San Raffaele, Milan Italy.

The inclusion criteria were the same as for the OTL-103-4 study.

The recommended dose was 2-20×10^6 CD34+ cells/kg and the actual dose administered was in the range 7.0-16.8 ×10^6 CD34+ cells/kg. The formulation used was the fresh formulation and the cell source was mPB (BM and mPB in 1 subject). While the formulation and cellular source is different from the intended commercial product, they were used in the non-clinical pharmacology studies. The conditioning regimen prior to GT was the same as in OTL-103-4 study.

Expanded use program (EAP): the EAP comprised Hospital exemption (HE) with 3 patients treated and the Compassionate use program (CUP) with 7 patients. The formulation used was the fresh formulation and the

cell source was mPB. The main inclusion criteria were consistent with the TIGET-WAS study. The median dose was 15.50×10^6 CD34+ cells /kg (range 11.2 - 26.4). The severity of disease at the time of TG seems lower in the EAP program than in the clinical studies, in line with exclusion criteria which were more stringent for the CUP part of the EAP.

Efficacy data and additional analyses

For study OTL-103-4, in the initial submission, the analysis of the primary efficacy set included 12 months data for 10/10 patients. Upon request from CAT/CHMP, the applicant has submitted the clinical data with a more recent data cut-off (DCO) (17 Jan 25) which allows for a 2-year follow-up for all 10 patients.

Main study OTL-103-4

The 10 participants were all male, age 1-9 years (median 2 years). The sample size of 10 patients was considered acceptable, in the protocol assistance received by CHMP, given the limited patient pool for the disease.

In terms of severity of disease, 9/10 subjects had at least one criterion of severe WAS (severe WAS gene mutation, or Zhu score >= 3.0) and a history of severe infections, while 10/10 had eczema and 7/10 had thrombocytopenia. A side-by-side comparison of baseline disease characteristics showed a considerable clinical benefit after Telethon003 treatment regardless of disease characteristics at baseline. There was no relation between Zhu score at baseline and efficacy results. Patients with WAS protein 'absent' and respectively Class 2 WAS mutation had slightly more events of severe infections and severe bleeding events in the post-Telethon003 treatment period, mainly before the Y3 timepoint.

All 10 participants completed at least 2 years of follow-up, with eight participants completing 3 years of follow-up. Median duration of follow-up was 5.0 years (range: 2.31–5.43 years). Six participants had completed the 5-year follow-up visit. Primary objectives and outcome: a considerable decrease in the annualized rate of severe infections events from 2.403 (95% CI: 1.5399, 3.5762) per person-year of observation (PYO) for the period within 12 months pre-GT to infusion to 0.200 per PYO in the 6–18 months post-Waskyra. and this despite cessation of prophylactic IgRT and antimicrobial treatment. The number of participants with severe infections decreased from 9/10 in the 12 months pre-GT to 1/10 at Y2 and 0/8 at Y3. It is also worth mentioning the marked decrease in the rate of hospitalizations for infections and bleeding events (secondary endpoint).

Similarly, there was a marked decrease in the rate of moderate and severe bleeding from 0.901 (0.41- 1.71) events per PYO in the pre-treatment phase to 0.300 (0.062; 0.877) in the 12 months and to 0.300 events per PYO in the first 12 months following Waskyra and to 0.200 in the Year 1-2 post-GT. No participant required platelet transfusions in the period after 6 months of FU, compared to 10.29 infusion per PYO in the 12 months pre-GT.

In this study, 2 patients with severe infections and 2 patients with moderate and severe bleeding events were reported in the >6 months post-GT. Also 9 and respectively 11 patients were reported post-treatment in the integrated analysis (with all studies included). In the updated analysis provided upon request from CAT/CHMP, it appeared that the rate of severe infections and respectively bleeding decreased considerably and steadily over-time, with only sporadic events after Year 3 (1 of each) that resolved with treatment. From the patient narratives it was agreed that there was no relation between degree of engraftment and events of serious infections or bleeding.

Secondary objectives: The overall survival was 100% at 24 months, and all patients are alive at the DCO (5 patients completed the Y5 visit).

Haematological reconstitution defined as ANC >500 cells/L was reached by 9 (90%) participants at D60 and 10/10 by D90 and was maintained throughout follow-up period.

Engraftment of gene-modified cells in peripheral blood at 6 months post-GT by VCN/cell has been reached by all evaluated participants and was maintained over-time. WAS protein expression as the median percentage of PB cells (platelets, lymphocytes, B-cells, T-cells, NK cells, and monocytes) expressing WA

SP increased after Telethon003 infusion steadily up to 6 months and remained well above baseline until the data cut-off.

However, failure of engraftment remains a potentially important risk after Waskyra infusion. This is addressed in the section 4.4. of the SmPC and in the RMP. In case of persisting neutropenia, G-CSF should be started to stimulate bone marrow recovery or earlier based on medical judgement.

As part of the therapeutic approach back-up autologous If failure of engraftment and neutropenia persist despite the use of granulocyte colony – stimulating factor, administration of the non-transduced back up autologous stem cells is recommended.

The median %LVV + BM-CFU was 51% (38%, 77%) (n=9) at D30 but decreased to 36% (10,72) (n=7) at Year 3 and 42.9% (10, 67), n=5 at Y5. The VCN/cell are stable overtime (Y1, Y3, Y5). At individual level, values decreased from D30 to Y3, and in one patient reached the threshold of 10% LVV+ at Y3. Lower values were observed at Y3 and Y5 than the other time-points, with values as low as 10% in 3 patients, while an increase was observed up to Y8. However, no relationship with a clinical outcome established. Of note, for the evaluated patients, no increase in infections or bleeding was observed in the Y5 to Y8 period.

Increase in platelet count, median platelet volume, and decrease in eczema severity were observed post-GT, however the number of subjects is too limited to draw meaningful conclusions. The Zhu score decreased considerably post-GT, with the few exceptions of patients who had a Zhu score of 5A at baseline and who presented flares of autoimmunity or auto-inflammation. This highlights the importance of treating WAS disease before installation of the autoimmune manifestations.

In addition, restoration of immunological function by T cell response to antigen stimulation and restoration of B cell function by immune response to vaccination was reported. The applicant presented an overview of vaccinations received reporting that in general the patients elicited an immune response following vaccination. In conclusion, apart for a warning against live vaccines administration, no other recommendations in the SmPC are warranted with regards to vaccination.

Supportive studies

TIGET-WAS:

The primary objectives and endpoints for the TIGET-WAS study were to evaluate the safety, long-term engraftment, and clinical efficacy assessed as overall survival, improvement of the immune function, and improvement of thrombocytopenia. Reduced rate of severe infections and bleeding episodes were secondary endpoints. All analyses were descriptive.

All 8 subjects were male, the median age was 2.2 years (range 1.1-12.4 years): 5/8 (62.5%) were < 5 years of age and 4/8 (50%) were < 24 months of age. In terms of disease characteristics at baseline, all participants had a Zhu score >3.0 and 2/8 (25%) participants had a score of 5.0A. WASP expression on

lymphocytes was 'absent' in 6/8 (75%) subjects, WAS mutations were class II (severe) in 7/8 (87.5%) subjects. Finally, all (100%) subjects had thrombocytopenia and infections at baseline, and petechiae was reported in seven [87.5%] participants.

The follow-up range in Study TIGET-WAS was 8.01 to 13.26 years (n=8) with a median of 11.1 years.

Overall survival at 8 years post-GT is 100%. The number of bleeding events per person years observed (PYO) decreased markedly from the period 12 months pre-GT (3.4) to >6 months post-GT (0.1). The number of severe infections per person years observed (PYO) decreased markedly from the period 12 months pre-GT (2.1) to >6 months post-GT (0.1). However, treatment with antimicrobials continued throughout the 8 years follow-up. The rate of platelet transfusion improved with treatment. The rate of hospitalization due to these events improved considerably starting 1-year post-GT. The rate of auto-immune manifestations was low both pre- and post-GT with no meaningful interpretation. The sustained engraftment of gene-modified cells (measured at VCN/cell) has increased post-GT, with the predefined thresholds being reached starting at D30 and, despite high variability over-time, was maintained throughout. The percentage of cells expressing the WAS protein also increased over-time, though data are not available for all subjects at all time-points. However, the restoration of T cell function was confirmed by positive response to vaccination rates and T-cell proliferation responses to stimulation with anti-CD3 antibody.

In summary the pivotal study results are corroborated by the results from the phase 1/2 supportive study which in addition provide assurance on the sustainability of the clinical benefit (with all the 8 patients from TIGET study having 8 years of FUP).

EAP

All (9) patients included in the EAP were male, 5/9 were < 5 years of age, 3/9 were > 11 years (14, 28, 35 years). All (9) patients had a Zhu score >= 3.0, and 9/9 had a severe WASP mutation.

The primary objective of this programme was to provide access to treatment to patients with unmet medical need.

Patients in the EAP (n=9) have a median follow-up of 5.948 years (range of 4.73–7.44 years for surviving patients) with seven patients having at least 5 years of follow-up.

Overall survival was 88.9% (8/9 patients) at 4 years post-GT. One adult patient died at 4.5 months post-GT from a pre-existing neurological condition unrelated to Telethon003.

The rate (event per PYO) of manifestations of WAS disease (severe infections, moderate and severe bleeding, and eczema) improved gradually post-GT compared to pre-GT period. The rate of platelet transfusion improved, however not meaningful comparison can be drawn as the rate in the 12 months pre-GT is not available. The rate of hospitalization improved considerably starting 6 months post-GT. The rate of auto-immune manifestations was low both pre- and post-GT with no meaningful interpretation. The sustained engraftment of gene-modified cells (measured at VCN/cell) has increased post-GT and was maintained in the range 0.5-2.0. The percentage of cells expressing the WAS protein also increased over-time and maintained through Year 6 of follow-up.

Integrated analysis

The applicant has presented the efficacy analysis as an integrated analysis comprising data from the 27 participants treated from the TIGET-WAS and OTL-103-4 studies and the EAP which allows for a more complete assessment of the clinical outcome. This was considered appropriate given the comparability of: studies population, methodology, and clinical outcomes, and comparability of fresh and the cryopreserved DP formulations. No formal comparison of results across the studies was conducted; however, consistency of data between studies is discussed, where relevant.

The critical issue for clinical efficacy is to appreciate exactly how the post-treatment responses were put into context by comparisons with pre-study data. The primary efficacy endpoints in the integrated efficacy analysis used the 12-month pre-treatment rates as the comparator. The applicant was requested to discuss the methodology applied to data collection and justify the reliability of the pre-study dataset. The applicant indicated that in order to ensure a reliable and standardised pre-study data collection, retrospective in nature, for all participants enrolled in the clinical studies (TIGET-WAS and OTL-103-4) and expanded access program (EAP), the Investigators/responsible physicians collected a full medical history, along with a review of the referring physician's medical notes. This included review of medical summaries from the referring healthcare providers, results of diagnostic tests, discharge letters and hospital notes from inpatients and outpatient's services who had followed the subject. It also included any other additional medically relevant information to document medical events, hospitalisations, diagnostic procedures including imaging and laboratory tests, surgical procedures and treatments the subject had in his history. Overall, the documentation collected was intended to cover the whole medical history of the subject from birth, in as much detail as possible. Data quality assurance was guaranteed through quality control provided in the form of data monitoring activity throughout the studies and EAP, aimed at verifying that the reported data were accurate, complete, consistent, and verifiable from source documents.

Data from these patients were contextualised with data on the outcomes of patients with WAS from a systematic literature search and from a worldwide independent survey of WAS patient outcomes. This approach was agreed in protocol assistance and generally followed.

At the most recent data cut off (17 Jan 2025), 26 surviving participants had a median follow-up of 6.816 years (range 2.31 to 13.26 years). The median age at the time of Telethon003 treatment was 2.2 years in TIGET-WAS, 1.984 years in OTL-103-4, and 3.841 years in the EAP.

All participants were symptomatic at enrolment and clinical features were comparable across both studies and the EAP, the only notable clinical difference being the lower incidence of moderate and severe bleeding events in the year preceding treatment in OTL-103-4 and the EAP compared with TIGET-WAS.
At baseline, 13/27 patients had a Zhu score of 3, 4/27 of 4 and 8/27 of 5A. The WAS protein expression was not absent (present, reduced, or revertant) in 11 subjects and 'absent' in 15. The WAS mutation was Class 1 in 5 subjects and Class 2 in 22 subjects.
The percentage of participants surviving at the end of follow-up for this analysis was 96% (95% CI: 82, 99%).
In the updated analysis with DCO 17JAN2025, for the integrated analysis, data is available for 26/27 patients for 2 years, 20/27 for 5 years, 8/27 for 8 years, 5/27 for 10 years and 1/27 for 13 years.

The rate of severe infections decreased substantially from 2.001 (95% CI: 1.5033, 2.6110) infections per person-year of observation (PYO) in the 12-month period before GT to 0.154 (95% CI: 0.0418-0.3931) infections per PYO in the 6–18 month and 0.120 in the 2-3 years post Waskyra and further to 0.046 at Y5.

The number of participants with severe infections decreased from 19/27 in the 12 months pre-GT to 3/26 at Y2 and 1/20 at Y5.

The rate of moderate and severe bleeding events decreased from 2.001 (95% CI: 1.5033, 2.6110) events per PYO in the 12 months before GT to 0.154 (95% CI: 0.0419-0.3942) per PYO in the 1-2 years, to 0.160 events per PYO in the 2-3 years post-Waskyra and to 0.018 (95% CI: 0.0004-0.0984) events per PYO in the >5-year period. The number of patients with moderate and severe bleeding decreased from 19/27 in the 12 months pre-GT to 10/26 in the 12 months post-GT, to 3/26 at Y2 and to 3/20 at Y5. By severity, the rate of severe bleeding events decreased from 0.889 (95% CI: 0.5698-1.3233) events per PYO in the 12 months before GT to 0.076 (95% CI:0.0092-0.2742) events per PYO in the 12 months and 0 in the 1-2 years following Telethon003, and the rate of moderate bleeding events decreased from 1.112 (95% CI: 0.7501-1.5870) events per PYO in the 12 months before GT to 0.721 (95% CI: 0.4341-1.1260) events per PYO in the 12 months and 0 in the 1-2 years following Telethon003. Post-treatment, there were 4 severe (Grade 3) bleeding events compared to 23 pre-treatment, and no Grade 4 events. Two occurred in the 6 months, 1 in the 3-5 years, and 1 in the more than 5 years post-treatment.

Regarding the moderate (Grade 2) bleeding events, after an initial increase in the first 6 months post-treatment, the rate of events decreased considerably: from 1.112 pre-treatment to 1.276 in the 1st 6 months, 0.154 in the 6-12 months, and 0.0146 in the 3-5 years post-treatment. For the >5 years FU the rate decreased to 0.

All participants achieved adequate engraftment of genetically corrected BM CD34^{+} and/or PB CD3^{+} cells by Day 34 post-Telethon003. Median time to gene- corrected cell engraftment was 32.0 days (range: 26–35 days). The median time to engraftment of PB CD3^{+} T cells alone was 91.0 days (range: 29–426 days).

The proportion of genetically corrected cells was 51% (18-77) at Day 30, 49% (17-85) at Y1, 39.45% (10-91) at Y3, 47.5% (10-100) at Y5, and 66.5% (30, 100) at Y8. The observed lower values at Y3 and Y5 are not explained however, do not correlate to other engraftment parameters or clinical outcome.

The analysis of cell populations transduced with the WAS gene (by means of assessment of VCN) showed a largely predominant transduced cell population >1-year post-GT derived from CD34^{+} cells which were not revertant, as any reversion would occur in precursor or mature cells and not in haematopoietic stem cells. Additionally, after GT the VCN in lymphoid cells was similar in both participants with and without previous revertant cells.

Overall, the magnitude of the efficacy is very high considering the annualised rate of moderate and bleeding events and the annualised rate of infections. This level of efficacy is also demonstrated by the overall survival rate of 26/27 surviving participants (95% CI: 82-99%) with a follow-up duration ranging from 2.31 to 13.26 years following treatment with Waskyra. One adult participant treated in the EAP died as a result of a fatal SAE unrelated to Waskyra.

Finally, a long-term follow-up (LTFU) PASS study (WAS-TLT003-01) has been agreed with the applicant. This study will include all patients treated during clinical development (clinical studies and EAP) and patients to be treated post-authorization to a total of 40 patients, and for a total duration of 15 years post-treatment, which is acceptable to CAT/CHMP. Characterization of efficacy is a secondary objective with multiple endpoints, consistent with the endpoints of the clinical studies. A subgroup analysis for patients >= 5 years of age will be performed.

Dose recommendation

The proposed minimum recommended dose is 7×10^6 $CD34^+$ cells/kg of body weight is considered acceptable. The dose range used in the clinical development is representative of the intended posology. This dose range is stated in the SmPC section 5.1. It is also recommended that the maximum volume of Waskyra to be administered should remain < 20% of the patient's estimated plasma volume.

Age / indication

The justification for the minimum age proposed by the applicant (6 months) in the indication was discussed in comparison with the age range treated (1.4-12.4 years). Post-transplant data showed positive outcomes in patients as young as 2 months of age at the time of HSCT, demonstrating the capacity for infants with WAS to tolerate a conditioning regimen and successfully engraft WASP-expressing hematopoietic stem cells. Six months was considered the anticipated earliest age at which GT may be performed following diagnosis of WAS, including the evaluation of eligibility for GT, leukapheresis, and DP manufacture. From a clinical standpoint, this can be agreed by CAT/CHMP. Therefore, the lower age limit of 6 months is agreed.

As expected, due to the short life expectancy of patients with WAS, very few patients were adults and only one adult patient was successfully treated in clinical development.

The integrated analysis, allowed for higher sample size for subgroup analysis by age (< 5 years and > 5 years) and severity of disease (Zhu score and WASP expression at baseline). At the time of analysis, 8/9 participants in the ≥5 years age group and all 18 participants in the <5 years age group were alive.

The applicant explained that the severity of the disease is mainly influenced by the WAS mutation and this may explain the apparently lower rate of severe infections, moderate and severe bleeding, and platelet count at baseline in the >= 5 years than in the younger subjects. In conclusion, available data from evaluable patients indicated that the efficacy in the >5y age group (n=8) is at least as good as in patients aged <5 years (n=18). No differences in other efficacy outcomes were observed among younger and older participants after Telethon003 administration. Overall, this is supportive of an extrapolation of the efficacy conclusions to participants outside the 1- to 12-year-old age group.

However, the risk of autoimmunity pre-GT, as well as the risk of incomplete reversal of clinical autoimmunity after gene therapy appeared higher in the age group >5 years. A differential response by age cannot be strictly excluded given the expected long history of immune suppression in those patients, high severity of disease, and complex disease history. Therefore, older patients may need to be carefully screened for conditions which may imply a higher risk for adverse reactions to conditioning, even with a reduced conditioning regimen before Telethon003 infusion. Nevertheless, it remains advisable to apply GT at younger age.

This is adequately mentioned in the SmPC section 4.4. It is advisable to administer gene therapy at younger age. The risk of autoimmunity pre-GT, as well as the risk of incomplete reversal of clinical autoimmunity after gene therapy appeared higher in the age group > 5 years. Also, older patients may need to be carefully screened for conditions which may imply a higher risk for adverse reactions to conditioning.

Gender

Given the genetic transmission, as expected, all patients were male. Literature describes very rare cases in female subjects, in whom a different pattern of disease may occur. However, it was agreed with the applicant that there is no scientific rationale for a gender dependent benefit/risk profile. In conclusion a broad indication to male and female patients is acceptable.

Special populations

Waskyra has not been studied in patients with HIV-1, HIV-2, active HBV, or active HCV. A screening will be perfomed. Prior administration as recommended in the SmPC. Patients with prior HSC transplant

Waskyra has not been studied in patients who have received a prior allogeneic HSC transplant with evidence of residual cells of donor origin. Treatment with Waskyra is contraindicated in these patients.

Patients with serious haematological disorders

Waskyra has not been studied in patients with evidence of myelodysplasia, cytogenetic alterations characteristic of myelodysplastic syndrome and acute myeloid leukemia, or other serious hematological disorders. Treatment with Waskyra is not recommended in these patients.

2.5.7. Conclusions on the clinical efficacy

The efficacy of Waskyra was assessed in an open label, single arm, phase 3, pivotal study in patients with severe WAS and no HLA identical related donor available for HSCT. The supportive data from 8 patients with a follow-up of 8 years in the phase 1/2 study are relevant for the extent and duration of the clinical outcome. In addition, 9 patients were treated in the EAP program. Overall, the integrated efficacy analysis comprised 27 patients treated with Waskyra.

All analyses were descriptive. The co-primary endpoints are clinically relevant and indicate a marked decrease in severe infections and moderate and severe bleeding episodes post-GT. Additional endpoints assessed the overall survival, efficiency of engraftment, restoration of immune response, and rate of hospitalization. Overall survival was 96.7%% and 100% patients reached adequate engraftment.

Finally, it is considered that efficacy has been demonstrated in the following population. *"for patients aged 6 months and older with Wiskott-Aldrich Syndrome (WAS) who have a mutation in the WAS gene for whom haematopoietic stem cell (HSC) transplantation is appropriate and no suitable human leukocyte antigen (HLA)-matched related haematopoietic stem cell donor is available"*

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

2.5.8. Clinical safety

2.5.8.1. Patient exposure

The population for this summary (Safety population) includes all participants who underwent $CD34^+$ cell harvest procedures in the clinical development program (Study TIGET-WAS, Study OTL-103-4, and the EAP).

This includes: All eight participants in Study TIGET-WAS; all ten participants in Study OTL-103-4 and all ten patients in the EAP.

Safety data were collected from this clinical development program from the date of screening up, and up to 3 years after Telethon003 infusion (36 months). Extended follow-up was then implemented up to 15 years after gene therapy (GT) infusion (see below).

AEs and SAEs were collected throughout 3 different study phases, as follows:

- Pre-treatment phase: from the date of screening up to and including the day before the on- treatment phase,

- On-treatment phase: from Day -22 (±1; day of rituximab administration) or the day of PBSC mobilization up to and including Day 1 (day of Telethon003 infusion),

- Post-treatment phase: from the day after Telethon003 infusion until the cut-off for analysis,

Pooled safety data collected from both clinical studies and the EAP are summarized below:

Table 19. Summary of number of participants with adverse events by treatment phase (Safety population)

	Pre-treatment[a] (N=28) n (%) [E]	On-treatment[b] (N=28) n (%) [E]	0–6 months (N=27) n (%) [E]	6–12 months (N=26) n (%) [E]	1–2 years (N=26) n (%) [E]	2–3 years (N=22) n (%) [E]	3–8 years (N=22) n (%) [E]	>8 years (N=8) n (%) [E]	Post-treatment (N=27) n (%) [E]
Any AE	28 (100) [348]	28 (100) [285]	27 (100) [571]	25 (96.2) [150]	26 (100) [260]	22 (100) [189]	21 (95.5) [562]	8 (100) [122]	27 (100) [1854]
AE of CTCAE Grade ≥2	25 (89.3) [127]	27 (96.4) [151]	27 (100) [280]	18 (69.2) [54]	25 (96.2) [118]	22 (100) [84]	20 (90.9) [250]	6 (75.0) [49]	27 (100) [835]
AE of CTCAE Grade ≥3	17 (60.7) [45]	23 (82.1) [71]	22 (81.5) [125]	5 (19.2) [7]	6 (23.1) [11]	7 (31.8) [10]	7 (31.8) [13]	2 (25.0) [3]	24 (88.9) [169]
AE related to treatment	0	0	0	0	0	0	0	0	0
Any SAE	5 (17.9) [7]	10 (35.7) [21]	18 (66.7) [35]	4 (15.4) [4]	4 (15.4) [5]	5 (22.7) [6]	6 (27.3) [8]	2 (25.0) [2]	20 (74.1) [60]
AE leading to study withdrawal	0	0	0	0	0	0	0	0	0
Treatment-related SAE	0	0	0	0	0	0	0	0	0
SAE leading to death	0	0	1 (3.7) [1]	0	0	0	0	0	1 (3.7) [1]
Events resolved[c], events (%)	124 (35.6)	155 (54.4)	556 (97.4)	140 (93.3)	249 (95.8)	177 (93.7)	482 (85.8)	91 (74.6)	1695 (91.4)
SAE related or probably related to CD34+ HSPC mobilization procedure	0	0	0	0	0	0	0	0	0
SAE related or probably related to leukapheresis	0	0	0	0	0	0	0	0	0
SAE related or probably related to conditioning	0	1 (3.6) [1]	2 (7.4) [3]	0	0	0	0	0	2 (7.4) [3]
SAE related or probably related to other study procedure	1 (3.6) [1]	5 (17.9) [5]	12 (44.4) [15]	1 (3.8) [1]	1 (3.8) [1]	0	0	0	13 (48.1) [17]

AE=adverse event; CD=cluster of differentiation; CTCAE=Common Terminology Criteria for Adverse Events; CUP=Compassionate Use program; E=number of events; eCRF=electronic case report form; HE=Hospital Exemption; HSPC=hematopoietic stem and progenitor cell; MedDRA=Medical Dictionary for Regulatory Activities; PBSC=peripheral blood stem cell; SAE=serious adverse event

a. From the date of screening up to and including the day before the on-treatment phase
b. From Day -22 or the day of PBSC mobilization if performed up to and including Day 1 (day of gene therapy)
c. Denominator for percentage of events resolved is the total number of events

Treatment related adverse events included adverse events reported as related or probably related on the eCRF.
In TIGET-WAS and 205030 (HE), SAE flag was not collected on relevant medical history and concomitant disease eCRF page. For CUP patients and OTL-103-4 participants only, pre-treatment events were collected on the adverse events form, so SAE flag was collected.
MedDRA Version 26.0 was used.

At the date of cut-off, the median duration of follow-up for the 27 participants in the safety population who were treated with Telethon003 was 5.67 years (range: 0.37–13.26 years (see below); excluding the participant who died 0.37 years after Telethon003 infusion, all participants have been followed for at least 1.19 years post-GT.

Table 20. Summary of duration of follow-up (Safety population)

		Telethon003
Number of participants (%)		28 (100)
Duration of follow-up (years)	n	27[a]
	Mean	6.577
	Standard deviation	3.2887
	Median	5.666
	Minimum	0.37[b]
	Maximum	13.26
Duration of follow-up in participants alive at data cut-off (years)	n	26
	Mean	6.816
	Standard deviation	3.1060
	Median	5.716
	Minimum	2.31
	Maximum	13.26

a. Patient PPD was not treated with gene therapy so had no follow-up
b. Patient PPD had PP years of follow-up before his death
Source: Table 2.7.4.1.2

For the 27 participants who received Telethon003, the median number of $CD34^+$ cells infused was 16.90 × 10^6 cells/kg.

Two formulations of Telethon003 drug product (DP) have been studied in the clinical development program:

- The fresh formulation of Telethon003 comprises the drug substance, as described above, re-suspended in saline and supplied in the final container closure system for administration.
- The cryopreserved formulation of Telethon003 comprises the drug substance, as described above, formulated in cryopreservation medium containing 5% dimethylsulfoxide and stored in the vapor phase of liquid nitrogen until administration, and supplied in the final container closure system for administration.

The cellular source material ($CD34^+$ HSPCs) used for Telethon003 manufacture was harvested either by direct aspiration from the BM or by mobilizing the cells from the BM and harvesting them by apheresis from the peripheral circulation (i.e., mPB). The $CD34^+$ HSPCs were mobilized from peripheral blood (PB) using granulocyte-colony stimulating factor (G-CSF; filgrastim or lenograstim) and the mobilizing immune modulator Plerixafor.

Then, twenty-seven of 28 (96.4%) participants received conditioning treatment, composed of rituximab, busulfan and fludarabine.

Of note, one patient a 25-year-old White male, did not receive conditioning or Telethon003 as insufficient autologous mobilized $CD34^+$ cells were harvested for obtaining the minimum target dose of $CD34^+$ cells required for DP manufacture.

To be in line with GT requirements for products using LVVs (*EMEA/149995/2008 rev.1; FDA, January 2020*), all participants in the clinical development program (Study TIGET-WAS, Study OTL-103-4, and the EAP) will be followed up for 15 years after treatment with Telethon003.

2.5.8.2. Adverse events

In the integrated safety data, the most frequently reported AE overall was petechiae, with 25 events in the pre-treatment phase, 28 events in the on-treatment phase, and 87 events in the post-treatment phase (35 of which were in the first 6 months post-GT).

Twenty AEs were reported in 15 (55.6%) participants in the first 48 hours after Telethon003 infusion. The most frequently reported AEs were petechiae (three events in three [11.1%] participants), hepatic enzyme increased (two events in two [7.4%] participants), and transaminases increased (two events in two [7.4%] participants).

Other AEs included anemia, cholestasis, decreased appetite, dermatitis, headache, hepatomegaly, mouth hemorrhage, nausea, otorrhea, respiratory syncytial virus test positive, sinus arrhythmia, viral upper respiratory tract infection, and vomiting (each occurring in one participant).

In the safety population, the MedDRA SOCs with the most frequently reported AEs were "*Infections and infestations*," with 59 events reported in 20 (71.4%) participants in the pre-treatment phase and 43 events in 22 (78.6%) participants in the on-treatment phase. In the post-treatment phase 499 events in this SOC were reported in 27 (100%) participants. Moreover, 110 of these events in 26 (96.3%) participants occurred in the first 6 months after the Telethon003 infusion.

The most frequently reported PTs in the SOC of "*Infections and infestations*" post-treatment included upper respiratory tract infection and rhinitis, which were generally of moderate severity.

In the "*Skin and subcutaneous tissue disorders*" SOC, 42 events were reported in 16 (57.1%) participants in the pre-treatment phase and 56 events in 22 (78.6%) participants in the on-treatment phase. In the post-treatment phase, 222 events were reported in 26 (96.3%) participants, with 100 of these events in 24 (88.9%) participants occurring in the first 6 months after Telethon003 infusion.

The AEs reported most frequently within this SOC included petechiae, eczema, and rash in the pre-treatment phase, on-treatment phase, and early post-treatment phase (0–6 months), which are consistent with events expected in patients with WAS prior to treatment and during participant recovery following conditioning and ongoing hematological and immunological reconstitution.

Of note, the most frequently reported dermatological AE in all phases was eczema. In the pre-treatment period, there were six events in four (14.3%) participants, eight events in six (21.4%) participants in the on-treatment phase, and 49 events in 15 (55.6%) participants in the post-treatment phase; 24 of the post-treatment events in 11 (40.7%) participants occurred in the first 6 months after Telethon003 infusion.

The most common Grade ≥3 AE was device related infection, with three events in the pre-treatment phase, four events in the on-treatment phase, and 11 events in the post-treatment phase.

2.5.8.3. Serious adverse events, deaths, and other significant events

The majority of participants in the safety population reported one or more SAEs, with 60 SAEs reported in 20 (74.1%) participants in the post-treatment phase. Within this phase, the majority of SAEs occurred in the first 6 months after Telethon003 infusion (35 events in 18 [66.7%] participants).

The SOC with the greatest number of SAEs in the post-treatment phase was "Infections and infestations", with 31 events reported in 15 (55.6%) participants. The majority of infection-related SAEs (19 out of 31) occurred in the first 6 months after Telethon003 infusion and could be attributed to the participants' post-

transplantation status, which was expected to cause a predisposition to infections owing to the ongoing immune reconstitution. Very few infections were reported in the subsequent periods.

The most frequently reported procedure-related SAE was device related infection, with one event in one (3.6%) participant in the pre-treatment phase and three events in three (10.7%) participants in the on-treatment phase. In the post-treatment phase, seven events were reported in six (22.2%) participants, with six of these events in six (22.2%) participants occurring in the first 6 months after Telethon003 infusion. Additionally, there was one event of device related infection in the 1–2-year period.

Serious Adverse Events related to Mobilization, Leukapheresis Procedure, Conditioning, or Other Study Procedures:

Concerning the whole procedure (including CD34+ mobilization, leukapheresis procedure, conditioning regimen, and other study procedures), no SAEs were considered related to the mobilization or leukapheresis procedure and 4 SAEs were considered related to or probably related to conditioning by the Investigator/treating physician. These SAEs were reported in:

- one (3.6%) participant in the on-treatment phase (shock). The SAE of shock (verbatim term: adverse reaction to rituximab administration [shock circulatory]) was considered by the treating physician to be related to rituximab.

- two (7.4%) participants in the first 6 months after Telethon003 infusion (neutropenia, thrombotic microangiopathy, and veno-occlusive liver disease). The SAEs of veno-occlusive liver disease and suspected thrombotic microangiopathy occurred in one participant and were considered by the Investigator to be related to conditioning following unexpected high exposure to busulfan. All three SAEs are listed in the busulfan SmPC.

Of note, no conditioning-related SAEs were reported beyond 6 months after Telethon003 infusion.

Twenty three (23) SAEs were considered related to or probably related to other study procedures by the Investigator/treating physician were reported in:

- one (3.6%) participant in the pre-treatment phase (one event)

- five (17.9%) participants in the on-treatment phase (five events)

and thirteen (48.1%) participants in the post-treatment phase (seventeen events), with 15 of these events in 12 (44.4%) participants occurring in the first 6 months after Telethon003 infusion.

Deaths

One death was reported in a 35-year-old White male, approximately 4.5 months after Telethon003 infusion (0.37 year) due to a deterioration of a pre-existing neurological condition (SAE preferred term: neurological decompensation; verbatim term: neurological deterioration). The patient had a medical history of spastic gait that was ongoing at screening, and a Grade 3 AE of neurodegenerative disorder (verbatim term: neurodegeneration with brain iron accumulation) with onset on Day -185. The event was considered unrelated to Telethon003 by the treating physician.

Significant events

One papillary thyroid cancer was reported in, a one patient, at 5 years post-treatment, who had a family history of non-malignant thyroid disease, referred to as Graves' disease. The event resolved with sequelae (i.e., iatrogenic hypothyroidism following total thyroidectomy). The Investigator did not consider this

neoplasia involving cells of not hematopoietic origin as related to Telethon003 but possibly related to conditioning and the long history of previous immune suppression in this patient.

2.5.8.4. Laboratory findings

Values for hematocrit, hemoglobin, and leukocytes decreased after the start of treatment, as expected due to conditioning. Hematocrit and hemoglobin subsequently returned to baseline levels by around Day 180 (6 months post-treatment), and leukocyte count returned to baseline levels by Day 90 (3 months post-treatment).

Laboratory abnormalities reported as AEs of CTCAE Grade ≥3 in two or more participants included febrile neutropenia, anemia, immune thrombocytopenia, neutropenia, eosinophilia, hepatic enzyme increased, hypokalemia, and hypoalbuminemia.

Overall, the low hematology values reported after treatment resolved by 6 months post-treatment.

Liver function test values such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) tended to increase transiently over a period of 3–6 months following conditioning and Telethon003 infusion. Liver function test values subsequently returned to baseline levels by around Day 180.

Laboratory abnormalities reported as AEs of CTCAE Grade ≥3 reported in two or more participants during the first 100 days after Telethon003 infusion, which were assessed in relation to the short-term safety and tolerability of the conditioning regimen, included hepatic enzyme increased, hypoalbuminemia, and hypokalemia.

No clinically meaningful shifts from baseline to post-treatment timepoints were noted for urinalysis parameters.

Clinically significant elevated body temperature values were reported as AEs of pyrexia. Pyrexia was the third most frequently reported AE.

Plots of individual participant profiles of physical growth (height and weight; excluding adult participants) over time are superimposed over World Health Organization standard growth curves of normal healthy boys aged up to 19 completed years in the general population.

2.5.8.5. Safety in special populations

- **Intrinsic Factors**

No notable differences in safety profiles were observed in the sub-analyses of data by intrinsic factors based on age or race.

- *Age Band (28 Days to <24 Months, ≥24 Months to <12 Years, 12 Years to <18 Years, ≥18 Years)*

No notable differences in the AE profile or SAE profile were observed when data were summarized by age band. However, due to the small number of adult participants and participants aged 12 years to <18 years, no robust conclusions can be drawn on potential differences between paediatric populations (<12 years) and these populations.

- *Age Group (<5 Years; ≥5 Years)*

There was no relevant differences in the AE and SAE profiles when data were summarised by age group.

- *Race*

No differences in the AE profile or SAE profile were observed when data were summarized by race.

- **Extrinsic Factors**

No differences in safety profiles were observed in the sub-analyses of data by extrinsic factors based on DP formulation or cell source.

- **Use in Pregnancy and Lactation**

Wiskott-Aldrich Syndrome is an X-linked recessive disorder that primarily affects male participants. No females, including pregnant and breastfeeding females, received Telethon003. (Please refer to non-clinical and clinical efficacy parts on this aspect).

Conventional studies on fertility are not applicable and no developmental and reproductive toxicity studies were conducted.

However, the presence of the WAS LVV in the testis of the mouse was assessed by quantitative polymerase chain reaction in the biodistribution study and no vector sequences were detected demonstrating that WAS LVV remained stably integrated within cells of human origin and did not mobilise to mouse tissues, including testes

Due to the lack of evidence for germline transmission and the limited distribution of Waskyra (i.e., primarily to hematopoietic tissues), no specific reproductive or developmental toxicity studies were considered warranted with WAS LVV-transduced HSPCs/Telethon003.

2.5.8.6. Safety observed by drug product formulation (Fresh or Cryopreserved)

Adverse events were summarized by DP formulation.

The most frequently reported SOC for all AEs with both the fresh and cryopreserved DP formulations was "*Infections and infestations*", followed by "*Skin and subcutaneous tissue disorders*", "*Gastrointestinal disorders*", and "*General disorders and administration site conditions*". "*Infections and infestations*" was the most frequently reported SOC for all SAEs with both the fresh and cryopreserved formulations.

Adverse events of pyrexia, epistaxis, petechiae and eczema occurred at higher frequency in participants treated with the fresh formulation, mainly during the first six months after Telethon003 infusion. However overall, data suggest that there was no relevant difference in the safety profile between the fresh and cryopreserved formulations.

- *Drug Product Cell Source (Bone Marrow or Mobilized Peripheral Blood)*

Adverse events in the Safety population were summarized by the DP cell source.

The most frequently reported SOC for all AEs with the BM-derived DP was "*Infections and infestations*", followed by "*Skin and subcutaneous tissue disorders*", "*Gastrointestinal disorders*", "*General disorders and administration site conditions*", and "*Investigations*". Similarly, the most frequently reported SOC for all AEs with the mPB-derived DP was "*Infections and infestations*", followed by "*Investigations*", "*Skin and subcutaneous tissue disorders*", and "*Gastrointestinal disorders*". For SAEs, "*Infections and infestations*" was the most frequently reported SOC for BM- and mPB-derived DP.

A higher number of AEs were reported to have occurred in >30% of participants in the BM-derived DP group compared with the mPB-derived DP group; however, it should be noted that the BM-derived DP population was small, therefore, no definite conclusions can be drawn.

Overall, these data do not indicate a relevant difference in the safety profile between the BM- and mPB-derived DP.

2.5.8.7. Immunological events

The risk that gene therapy derived WASP could induce unwanted immune responses in treated patients was investigated during the Telethon003 clinical development programme. Adequate screening and confirmatory assays were implemented to identify an immune response against WASP in addition to clinical monitoring for immune mediated adverse events. Participants were administered Telethon003 in hospital and remained in hospital until they had undergone haemopoietic reconstitution. Clinical monitoring for immune responses to Telethon003 was conducted throughout hospital admission and then were assessed at outpatient follow up.

No immune response to the LVV or the transgene was observed in any of the subjects enrolled in studies with Telethon003. Monitoring of anti-WASP and anti-Human immunodeficiency virus (HIV) protein p24 antibodies every 6 months for the first year and then once a year during the clinical trial follow-up period did not reveal any antibodies to WASP and/or to p24.

Overall, the immune-mediated events reported were expected considering the background disease of subjects. No events of infusion-related reaction, delayed type hypersensitivity or immune complex mediated reactions were reported that were considered related to Telethon003.

None of these events was classed as an SAE.

At last, no participant generated a detectable antibody response to WASP.

2.5.8.8. Safety related to drug-drug interactions and other interactions

Since Telethon003 is a gene therapy medicinal product, the standard program of pharmacokinetic and pharmacodynamic drug interaction studies for a new chemical entity is not applicable, and thus no drug interaction studies were conducted.

2.5.8.9. Discontinuation due to adverse events

There were no AEs leading to participant withdrawal from the clinical studies or from the EAP.

Telethon003 was administered as a single infusion and due to the nature of the medicinal product it could not be discontinued

2.5.8.10. Updated safety data (cut of 17 Jan 25)

Updated safety data provided during the assessment upon request, which includes the data of Study OTL-103-4 with a data cut-off date of 17 January 2025, showed no change in the overall risk/benefit assessment.

Indeed:

- No new AEs of Grade 4 (life-threatening) were reported;
- No new deaths were reported;

- No new SAEs were reported;
- No new cases of malignancy were reported;
- No new immune-mediated AEs were reported;
- No change in trend of clinical laboratory abnormalities or vital signs was reported;
- No AEs related to Telethon003 were reported;
- No change in the overall profile of AEs was reported;
- No participant generated a detectable antibody response to WASP;
- No participant had a positive test for RCL;
- All participants were negative for ACP;

One new non-serious severe AE (Grade 3) of abdominal pain was reported in the 0-6 months follow up period and considered related to conditioning by the investigator.

Besides, newly reported AEs were mainly in the SOC of infections / infestations (30 new AEs) and Investigations (9 new AEs). The vast majority of new reported AEs were of mild (n=25) or moderate (n=37) severity.

2.5.9. Discussion on clinical safety

The applicant discussed the safety data collected in the safety population, that includes all participants who underwent $CD34^+$ cell harvest procedures in the clinical development programme (28 participants in total). This programme consists of 3 clinical studies: Study TIGET-WAS (8 participants), Study OTL-103-4 (10 participants), Expanded Access Programme (10 participants). Clinical safety data were then pooled (integrated safety data).

All patients but one (who did not receive the conditioning regimen as an insufficient number of mobilized $CD34^+$ cells could be harvested) received a single infusion of Telethon003, with a median number of $CD34^+$ cells infused of 16.90×10^6 cells/kg (range: 7.0 to 30.9×10^6 $CD34^+$ cells/kg). Transduction efficiency (percentage of gene-transduced CD34+ cells in the DP) was >80% in 21 of 27 (77.8%) participants. Median VCN per cell across the 27 participants was 2.30 VCN/cell.

Treatment with Telethon003 was preceded by haematopoietic stem cell collection through peripheral blood mobilisation with G-CSF with or without Plerixafor followed by apheresis, pre-treatment with rituximab (anti-CD20 monoclonal antibody) and reduced intensity conditioning of busulfan and fludarabine.

Throughout the 2 clinical studies, safety data were collected continuously from the date of screening up, and up to 3 years after Telethon003 infusion (36 months) at pre-defined time points, and were defined by the following safety endpoints : Adverse events (AEs) (including Summary of AEs, Serious adverse events (SAEs) and deaths, Pre-defined groupings of AEs), Safety laboratory evaluations (haematology, chemistry, urinalysis, liver function tests), Immune response to transgene, Vital signs, Exposure to replication-competent lentivirus (RCL), Abnormal clonal proliferation (ACP).

Extended follow-up was then implemented up to 15 years after gene therapy (GT) infusion, according to GT recommendations for products using LVVs. The median follow-up duration was 5.716 years (range: 2.31–13.26 years) for the 27 participants who were treated with Telethon003 and alive at the date of cut-off of 17

January 2025. Twenty-six (92.9%) participants completed the Year 1 visit, 22 (78.6%) completed the Year 2 and Year 3 visits, 15 (53.6%) completed the Year 5 visit, 8 (28.6%) completed the Year 8 visit, 5 (17.9%) completed the Year 10 visit and one (3.6%) completed the Year 13 visit. Excluding the participant who died 0.37 years after Telethon003 infusion, all participants have been followed for at least 2.31 years post-GT.

- Adverse reactions related to Telethon003 drug product formulations:

The WAS LVV is similar to the vector used for the manufacture of an already authorised medicinal product. Both vectors use the same lentiviral backbone, the only difference between the WAS and the MLD LVV is the expression cassette containing the promoter and the transgene. Further, an analysis of AEs per formulation fresh or cryopreserved) was provided. The data indicated that overall, no relevant difference in the safety profile could be concluded between the two formulations

Adverse Reactions related to medical device for administration

- The most frequently reported procedure-related SAE was device related infection.

Adverse Events and Serious Adverse Events:

AEs

The applicant has allocated the adverse events per pre-specified treatment phases: "*Pre-treatment phase*" from the date of screening up to and including the day before the on-treatment phase, "*On-treatment phase*" from Day -22 (±1; day of rituximab administration) or the day of PBSC mobilization up to and including Day 1 (day of Telethon003 infusion), and "*Post-treatment phase*" from the day after Telethon003 infusion until the latest cut-off of January 2025 for analysis. This is acceptable.

The most frequently reported AE overall was petechiae, with 87 events in the post-treatment phase (35 of which were in the first 6 months post-GT). Then, the second most reported AE was "*Upper respiratory tract infection* » with 140 events in the post-treatment phase.

Overall, the most frequently reported MedDRA SOC was "*Infections and infestations*", with 59 events reported in 20 (71.4%) participants in the pre-treatment phase, 44 events in 22 (78.6%) participants in the on-treatment phase and 523 events in 27 (100%) participants in the post-treatment phase, especially in the first 6 months after the Telethon003 infusion.

The third most frequently reported AE was pyrexia, with 93 events in the post-treatment phase.

Of note, at the time of analysis, 91.9% of post-treatment AEs had resolved.

The majority of post-treatment Grade 4 AEs (10 of 12 events) occurred in the first 6 months after Telethon003 infusion and included: neutropenia (three events in three [11.1%] participants) and acute respiratory distress syndrome, autoimmune neutropenia, bacterial sepsis, disseminated intravascular coagulation, electrolyte imbalance, neutrophil count decreased, and pneumonia aspiration (each reported in one [3.7%] participant).

SAEs

The majority of participants had SAEs, with 60 events reported in 20 (74.1%) participants in the post-treatment phase. Within this phase, the number of SAEs decreased over time, with 35 of the 60 events reported in the first 6 months after Telethon003 infusion. All of the SAEs, except for one fatal case, had resolved by the time of data cut-off.

The SOC with the greatest number of SAEs in the post-treatment phase was "*Infections and infestations*", with 31 events reported in 15 (55.6%) participants. The majority of infection-related SAEs (19 out of 31) occurred in the first 6 months after Telethon003 infusion.

Those observations are in line with what was expected considering the participants' post-transplantation status, which can cause a predisposition to infections owing to the ongoing immune reconstitution.

Two SAEs occurred without a relationship with conditioning being excluded : one death was reported in a patient, approximately 4.5 months after Telethon003 infusion (0.37 year) due to a deterioration of a pre-existing neurological condition, and one papillary thyroid cancer was reported in one patient, at 5 years post-treatment, who had a family history of non-malignant thyroid disease, referred to as Graves' disease (verbatim term: autoimmune hyperthyroidism). The event resolved with sequelae (i.e., iatrogenic hypothyroidism following total thyroidectomy). Both SAEs were considered unrelated to Telethon003 by the treating physician/Investigators. This is considered agreeable by CAT.

The majority of participants had AE(s) of CTCAE Grade 3 or higher, but these were all attributable to the protocol procedures, the underlying disease, and/or other medical conditions. The majority of the post-treatment severe events occurred during the first 6 months after Telethon003 infusion, while haematological reconstitution was ongoing. None of these reactions were related to Waskyra.

- Serious Adverse Events related to Mobilization, Leukapheresis Procedure, Conditioning, or Other Study Procedures:

The role of conditioning and other study procedure (such as: central venous catheter (CVC)) is also, and more largely of the whole treatment procedure (including mobilisation and leukapheresis as well) needs to be taken into account in the assessment of the safety data.

Infections and bleeding related to the use of CVCs have been reported in clinical trials. It is recommended that patients should be closely monitored for potential infections and catheter-related complications. This is adequately described in the SmPC sections 4.4 and 4.8.It was expected that the post-treatment phase would gather the greatest number of SAEs (60 SAEs reported in 20 [74.1%] patients), when compared to the pre-treatment phase (7 SAEs reported in 5 [17.9%] patients) and the on-treatment phase (21 SAEs reported in 10 [35.7%] patients). In other terms, near 70% of all the SAEs collected throughout the 3 treatment phases occurred in the post-treatment phase, with almost 40% of all the collected SAEs occurred within the 6 first months after Telethon003 infusion (35 events in 18 [66.7%] patients).

The SOC with the greatest number of SAEs in the post-treatment phase was "*Infections and infestations*", with more than half infection-related SAEs (19 out of 31) that occurred in the first 6 months after Telethon003 infusion. Interestingly, the Preferred Terms (PTs) "*device related infection*" and "*device related sepsis*" represent almost half of all the observed "*Infections and Infestations*" that occurred during the 6 first months after Telethon003 infusion (9 out of 19 SAEs reported).

The applicant explains this higher occurrence of SAEs "*Infections and infestations*" (SOC), especially within the 6 first months after Telethon003 infusion by the participants' post-transplantation status, which was expected to cause a predisposition to infections owing to the ongoing immune reconstitution. Besides, the necessary installation of central venous catheters for central venous access for busulfan and fludarabine conditioning and for the infusion of Telethon003 is also associated with a risk of device related infection.

In conclusion, no adverse reaction was related to Waskyra, but adverse reactions were related to the medical device and the conditioning treatment. However, no conditioning-related SAEs were reported beyond 6

months after Telethon003 infusion. No SAEs were considered related to the mobilization or leukapheresis procedure.

The SmPC section 4.2 and section 4.4. of the SmPC adequately describes the need for monitoring and risks associated with the pre-waskyra treatment procedures and refers to the SmPC of therapies used for mobilization, pretreatment, and preconditioning.

Overall summary of AEs and SAEs

Finally, in the target population, the analysis of the integrated safety data regarding the occurrence of AEs and SAEs do not suggest the emergence of any safety concerns related to the Telethon003 in one hand, and do not suggest the emergence of new safety concerns related to the whole therapeutic strategy, in other hand.

In study OTL-103-4, 5 patients had significantly higher exposures to busulfan than the target range of cumulative AUC of 48,000 ng/mL·h (±10%) and presented multiple serious adverse events.

In particular, it was concluded by the applicant that the occurrence of two SAEs (one event of veno-occlusive liver disease and one event of suspected thrombotic microangiopathy in one patient) was due to this higher exposure, this is agreed with CAT/CHMP. This unexpected high exposure occurred despite corrective measures to decrease busulfan dosing and pharmacokinetic monitoring as per protocol. Nevertheless, it is important to state that the root cause of the busulfan overexposure could not clearly be identified and may be multi-factorial. Following these events, mitigation measures were implemented by the applicant into the protocol during the clinical study. They are considered appropriate, as busulfan overexposures were prevented from re-occurring in subsequent patients.

In conclusion, the CAT/CHMP considered that information to strictly adhere to the target AUC of busulfan and monitor its PK while dosing would be necessary. This has been added in the SmPC sections 4.2 and 4.4.

In addition, a monitoring of the blood count has been introduced in section 4.2 and 4.4 of the SmPC for at least 6 weeks after infusion until recovery of the haematopoiesis and infections managed according to standard guidelines and clinical judgement.

The death of one patient was considered not related to the study drug by the investigator, but resulting from deterioration of a pre-existing neurological condition , that worsened approximately 4.5 months after Telethon003 infusion. No causal relationship of the fatal event with Telethon003 was established, this is agreed by CAT/CHMP.

No AEs or SAEs were assessed as related or probably related to Telethon003, which is an autologous product.

In summary, the analysis of the integrated safety data regarding the occurrence of AEs and SAEs with separate analysis for pre- treatment and post-treatment phase do not suggest the emergence of any particular safety concerns related to the Telethon003This is correctly reflected in section 4.8 of the SmPC.

Hypersensitivity and infusion-related reactions

Dimethylsulfoxide (DMSO), one of the excipients of Waskyra, is known to possibly cause serious hypersensitivity reactions, including anaphylaxis. The need to closely observe patients during and after infusion has been addressed in the SmPC sections 4.2 and 4.4.

Immunogenicity of Telethon003, Replication Competent Lentivirus, Abnormal clonal proliferation, malignancy:

No participant generated a detectable antibody response to WASP. Results of RCL testing were negative in all participants. The molecular analysis of the LVV integration sites in BM and PB samples collected after treatment indicated polyclonal hematopoietic reconstitution and the absence of clonal expansion or enrichment for proto- oncogenes in all participants. Overall data indicate stable engraftment of vector-marked clones long-term.

- Special populations

No relevant differences in safety profiles were observed in the sub-analyses of data by race, age, or CD34+ HSPC harvest source.

Finally, it is acknowledged that there is a risk associated with previous treatment with haematopoietic stem cell gene therapy or with allogeneic HSC transplant with evidence of residual cells of donor origin (mixed chimerism).

In case of the presence of **residual donor T cells** there is increase in the risk that the T cells will reject the drug product (due to HLA disparity); this will require a different conditioning regimen to deplete the residual T cells.

Therefore, a contra-indication has been implemented in patients with previous treatment with haematopoietic stem cell gene therapy or with allogeneic HSC transplant with evidence of residual cells of donor origin.

- Laboratory findings:

Overall, the laboratory data were consistent with a population that has a primary immunodeficiency and needs to undergo conditioning to receive HSCT. The CAT concluded that none of the laboratory changes could be attributable to Waskyra

Risk associated with germline transmission

Due to the lack of evidence for germline transmission and the limited distribution of Waskyra (i.e., primarily to hematopoietic tissues), no specific reproductive or developmental toxicity studies were considered warranted with WAS LVV-transduced HSPCs/Telethon003.As conditioning regimens using busulfan are known to be associated with potentially irreversible infertility, patients and their parents/carers should be advised about the options for cryopreservation of spermatogonia stem cells or ovarian tissue prior to application of conditioning Moreover, the busulfan SmPC and PL recommend that women of childbearing potential and men capable of fathering a child must use reliable barrier contraception. A reference to the SmPC of the conditioning regimens such as busulfan has been mentioned in the SmPC.

Risk of insertional oncogenesis

There is a theoretical risk of leukaemia or lymphoma after treatment with Waskyra. In the event a malignancy is suspected or confirmed, the marketing authorisation holder must be contacted for detailed instructions on sample collection for vector integration-site analysis (ISA) and clonal proliferation testing.

The long term follow-up registry study (PASS) will monitor this long-term risk over 15 years.

In conclusion, based on the integrated analysis observed in all 27 patients treated with Waskyra (data cut off on 17 January 2025), it is concluded that no ADR can be attributable to Waskyra but to the pre-treatment procedures and related therapies. Experience gained with other autologous cell therapies and the autologous

nature of the medicinal product, provides additional external support to the safety profile. Further, while considering the limited number of subjects, the 27 patients represent more than 25 % of the overall EU patient population. Taking into account the fact that 8 patients were followed up to 8 years, the safety profile is considered sufficiently characterised.

In addition, the OTL study is still ongoing, and 5 years data will be provided in 2028. It is mentioned in the RMP as a category 3 study.

However, the long-term safety will need to be further substantiated, in the post marketing setting, in particular in relation to the risk of oncogenesis. Therefore, a follow up PASS registry of 15 years follow-up for 40 patients has been agreed. The final report will be submitted in 2046. This missing information on long term safety is adequately described in the RMP (see later).

2.5.10. Conclusions on clinical safety

Upon CAT/CHMP request, the applicant provided additional safety data up to 17 Jan 2025, including an updated Integrated Summary of Safety that showed that there was no change in the overall risk/benefit assessment based on the provided updated safety data. In terms of exposure, all 26 surviving patients now have completed at minimum 2 years follow up after Telethon003 infusion. Twenty-four (24) participants completed the Year 3 visit, 20 patients completed the Year 5 visit and 8 participants completed a Year 8 visit.

As gene therapy administration is an intrinsic part of a whole therapeutic strategy, other procedures including $CD34^+$ mobilization, leukapheresis, reduced intensity conditioning using busulfan and fludarabine, and insertion of a central venous catheter, with well-known side effects, were considered in the overall safety assessment.

Overall, the safety findings, in terms of nature and frequency, were consistent with what was expected in this clinical setting, with WAS patients who undergone RIC, and subsequent haematological reconstitution following autologous HSCT. The first 6 months post-treatment are particularly at risk due to the participants' increased vulnerability to infections after conditioning, and while the reconstitution of the participants' immune systems is ongoing, which was reflected in the collected safety data.

Considering the integrated safety data collected in the safety population, Telethon003 was well tolerated, with a safety profile that appears to be acceptable: no AEs or SAEs related to the product were observed.

Albeit longer term data (8 years) are available for a very small number of patients (8), no engraftment failure, no malignancy due to insertional oncogenesis, no patient positive for replication competent lentivirus, no patient with a detectable antibody response to WASP were observed.

However, even if the safety profile of Telethon003, and more extensively of the whole therapeutic strategy seem acceptable, further long-term safety data are needed to substantiate the long-term safety profile of Telethon003, and to evaluate more specifically the occurrence of potential malignancies due to insertional oncogenesis in relation to the integrative lentiviral viral vector.

Educational materials for HCP, safety tools, patients career card have been agreed as part of the RMP in order to provide further detailed information about Waskyra administration and the overall therapeutic strategy.

While albeit proportionate to the rarity of the disease, the number of patients treated with Waskyra is limited (N=27), a PASS study will provide further clinical experience with 40 patients being targeted.

Finally, as required for gene therapy medicinal products, a 15-year long -term follow up is necessary to characterise the long-term safety profile of Waskyra. Thus, the CAT considers the following measures necessary to address issues related to safety:

PASS study

To characterise the long-term safety and efficacy of Waskyra, the MAH shall conduct a prospective study based on data from a registry, according to an agreed protocol "A Long-term Follow-up Study for Subjects Previously Treated with Autologous ex vivo Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome (WAS)". The study will enrol 40 patients. The final report will be submitted in December 2046.

Additionally taking into account the nature of the medicinal product and its overall therapeutic management requiring pre-treatment and reduced conditioning, educational materials have been agreed for Health car professionals and patients.

The CHMP endorses the CAT conclusion on clinical safety as described above

2.6. Risk Management Plan

2.6.1. Safety concerns

The following summary of safety concerns have been identified and are inserted in the RMP:

Table 21. Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Malignancy due to insertional oncogenesis Engraftment failure
Missing information	Long-term safety data

2.6.2. Pharmacovigilance plan

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 - Required additional pharmacovigilance activities				
A Long-term Follow-up Study for subjects Previously Treated with Autologous ex vivo Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome (WAS) **Approved, Initiated**	To characterize the long-term safety and efficacy of the gene therapy treatment	• Safety and tolerability as measured by adverse event (AE) and serious adverse event (SAE) related to gene therapy, including: • insertional mutagenesis and oncogenesis (blood and solid malignancies) • transgene immunogenicity • development of replication-competent lentiviruses (RCL) • insertion site analysis (ISA) • to evaluate the overall survival (OS) • to evaluate the overall survival up to 15 years of follow-up post treatment with gene therapy • to evaluate the risk of engraftment failure	• Interim analysis	Protocol submission: Within 3 months of the Commission Decision Interim analyses: Every two years. Final CSR: 31 December 2046
			• Final study report	

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 3 - Required additional pharmacovigilance activities				
A Single Arm, Open Label Clinical Study of Hematopoietic Stem Cell Gene Therapy with Cryopreserved Autologous CD34+ Cells Transduced with Lentiviral Vector encoding WAS cDNA in Subjects with Wiskott-Aldrich Syndrome (WAS).	Primary: to evaluate the clinical efficacy of the cryopreserved formulation of Telethon003 at 12 months for bleeding events	• Malignancies due to insertional oncogenesis • Engraftment failure • Long-term safety data	• Interim analysis	Dec 2021 July 2024 Apr 2025

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Ongoing	and from 6 to 18 months for severe infections Secondary: to evaluate the overall survival at 12, 24 and 36 months to evaluate the engraftment at 6 months to evaluate the safety of treatment with Telethon003 to evaluate the biological efficacy of the cryopreserved formulation of Telethon003 at 12 months, 2 years and 3 years to evaluate the clinical efficacy of the cryopreserved formulation of Telethon003 at 2 and 3 years to evaluate sustained engraftment of the cryopreserved formulation of Telethon003 at 2 and 3 years to evaluate the immunological function after treatment with Telethon003 up to 3 years to evaluate the effect of Telethon003 on health-related quality of life at 1, 2 and 3 years		• Final study report	Jun 2028

2.6.3. Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Malignancy due to insertional oncogenesis (Important potential risk 1)	Routine risk minimisation measures: SmPC section 4.4 where advice is given on theoretical risk of insertional oncogenesis. • Warning that Waskyra may theoretically cause leukaemia or lymphoma with instructions on tissue/ blood sample collection if malignancy occurs in SmPC section 4.4 • Information that there have been no cases of leukaemia or lymphoma in clinical studies in SmPC section 4.4. • Information that no abnormal or malignant growth of transplanted cells or hematopoietic tumours were found in a study in mice in SmPC section 5.3 • Information that no patients have developed leukaemia or lymphoma in PL section 2 • Warning that the patient will be asked to enrol in follow up study for up to 15 years and will be monitored for any signs of blood cancer because of the theoretical cancer risk in PL section 2 Additional risk minimisation measures: • Educational/safety advice tools for healthcare professionals • Educational/safety advice tools for patients • Provision of Patient Cards Other routine risk minimisation measures beyond the Product Information: • Legal status: Medicinal product subject to restricted medical prescription	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Study OTL-103-4 • A Long-term Follow-up Study for Subjects Previously Treated with Autologous ex vivo Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome (WAS)

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Engraftment failure (Important potential risk 2)	Routine risk minimisation measures: • Instructions to obtain a $CD34^+$ stem cell back-up for use as rescue treatment in SmPC section 4.2 • Information that no patients failed to engraft bone marrow in SmPC sections 4.4 and 5.1 • Information on identifying this potential risk and instructions for its resolution in Section 4.4 of the SmPC • Information that the doctor will collect a stem cell backup sample in case Waskyra fails to engraft in SmPC section 4.2, and PL section 3 • Warning that in case of cytopenia symptoms, red blood cells and platelet counts should be monitored until recovery is achieved in SmPC section 4.4 • Guidance that in case of engraftment failure, the non-transduced back-up cells should be infused according to local standards in SmPC section 4.4 • Guidance that if the modified stem cells do not take hold (engraft) in the patient's body, the doctor may give an infusion of the backup original stem cells in PL section 2 Additional risk minimisation measures: • Educational/Safety advice tools for healthcare professionals • Educational/Safety advice tools for patients Other routine risk minimisation measures beyond the Product Information:	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Study OTL-103-4 • A Long-term Follow-up Study for Subjects Previously Treated with Autologous ex vivo Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome (WAS)

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	• Legal status: Medicinal product subject to restricted medical prescription	
Long-term safety data (Missing information 1)	Routine risk minimisation measures: • Information on the duration of patient follow-up in the clinical studies in SmPC section 5.1 • Guidance that patients are expected to/ will be asked to enrol in a follow-up study for up to 15 years in SmPC section 4.2 and PL section 2 Additional risk minimisation measures: • Educational/safety advice tools for healthcare professionals • Patient and parent/carer information pack • Risk minimisation control programme Other routine risk minimisation measures beyond the Product Information: • Legal status: Medicinal product subject to restricted medical prescription	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Study OTL-103-4 • A Long-term Follow-up Study for Subjects Previously Treated with Autologous ex vivo Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome (WAS) •

2.6.4. Conclusion

The CAT considers that the risk management plan version 0.6 is acceptable.

The CHMP endorses the CAT conclusion on the RMP as described above.

2.7. Pharmacovigilance

2.7.1. 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.

2.7.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD).

2.8. Product information

2.8.1. 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.*

2.8.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Waskyra 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,
- It is a biological product that is not covered by the previous category and authorised after 1 January 2011,
- It has a PASS imposed either at the time of authorisation or afterwards; [REG Art 9(4)(cb), Art 10a(1)(a), DIR Art 21a(b), Art 22a(1)(a)],

Therefore, the summary of product characteristics and the package leaflet include 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

Wiskott-Aldrich Syndrome (WAS) is an X-linked primary immune deficiency and platelet disorder, characterised by thrombocytopenia and associated bleeding, eczema, recurrent infections, with increased susceptibility to autoimmunity, and lymphoreticular malignancies (lymphomas, leukaemias, and myelodysplasias).

Wiskott-Aldrich Syndrome is a life-threatening illness associated with a severely reduced life expectancy and median survival of 14.5 years without definitive intervention. The majority of patients fail to reach adulthood.

3.1.2. Available therapies and unmet medical need

Allogeneic HSCT, in which stem cells are obtained from a matched or mismatched, related, or unrelated donor, is considered the only potential disease-modifying treatment for WAS. When successful, HSCT leads to

an improvement in platelet count and immune function and to the resolution of eczema. However, HSCT is often associated with severe short- and long-term complications including conditioning-related toxicity, graft failure and GvHD.

Only about 25-30% of patients have access to a matched related donor (MRD), and availability of a 10/10 matched unrelated donor is largely dependent on race and haplotype. In addition, outcomes for patients >5 years of age are still less favourable than those of younger patients, placing an urgency on early treatment.

Conventional supportive treatments, which only manage the clinical manifestations of the disease, include platelet transfusions, anti-fibrinolytic agents or off-label TPO receptor agonists, antimicrobials, IgRT, and immunosuppressive drugs, corticosteroids, and anti-CD20 monoclonal antibody rituximab for the management of autoimmune diseases.

Splenectomy can be an effective treatment for thrombocytopenia, but it does not correct the underlying defect in platelets and carries a significant long-term risk of bacterial sepsis and may not be effective in the setting of autoimmune thrombocytopenia.

Therefore, there is an unmet medical need for an effective treatment in patients with WAS, especially in case of absence of HSCT donor.

3.1.3. Main clinical studies

The main evidence of efficacy was based on the phase 3 study OTL-103-4, a single arm, open label, non-randomized study (N=10) for evaluation of Waskyra in subjects with WAS disease. The co-primary endpoints were reduction in rate of severe infections from 12 months pre-GT to 6-18 months post-GT and respectively reduction in the rate of moderate and severe bleeding events from 12 months pre-GT to 12 months post-GT. Other secondary endpoints included overall survival, engraftment, and reduction in the hospitalization rate.

This pivotal study was supported by the TIGET-WAS phase 1/2 study and by an expanded use program (EAP) comprised of hospital exemption (HE) and compassionate use program (CUP).

In total 27 patients have been treated with Waskyra in the clinical programme. Two formulations, fresh (with both bone marrow and mobilised peripheral blood (mPB) as source of cells) and cryopreserved (mPB source) were used in clinical development. While those TIGET-WAS and the EAP programme were performed with the fresh formulation, the OTL-103-4 study was performed with the intended commercial cryopreserved formulation.

Conducting a controlled study was considered not feasible as the only other potentially disease-modifying treatment for WAS is HSCT, which carries a significant risk of mortality and severe complications, particularly when participants are aged ≥5 years or do not have a fully matched donor. Therefore, the CHMP recommended the applicant to compare clinical endpoints with data collected in the 12 months pre-study and from literature in order to address the lack of a parallel control group.

The median age at the time of Waskyra treatment was 2.2 years in TIGET-WAS, 1.984 years in OTL-103-4, and 3.841 years in the EAP. In the total population for the integrated analysis, the subjects' age ranged from 1.0 year to 35.1 years**.** Eighteen participants were aged < 5 years and nine were ≥ 5 years, of which two adults, both enrolled in the EAP.

The applicant presented the efficacy analysis as an integrated analysis comprising data from the 27 participants treated from the TIGET-WAS and OTL-103-4 studies and the EAP which allowed for a more

complete assessment of the clinical outcome. This was considered appropriate given the comparability of studies population, methodology, and clinical outcomes, and comparability of fresh and the cryopreserved DP

3.2. Favourable effects

The co-primary endpoints showed a considerable decrease in the annualized rate of severe infections events from 2.403 (95% CI: 1.5399, 3.5762) per person-year of observation (PYO) for the period within 12 months pre-GT to 0.200 (95% CI: 0.0250, 0.7454) per PYO in the 6-18 months post-GT.

Similarly, a marked decrease in the rate of moderate and severe bleeding from 0.901 (0.41- 1.71) events per PYO in the pre-treatment phase to 0.30 (0.062; 0.877) in the 12 months post-GT was shown.

Engraftment of gene modified cells, WAS protein expression at 6 months, and increase in platelet count as well as a decrease in eczema severity, in hospitalization rate and in rate of platelet transfusions were observed at 12 months post gene therapy.

These effects were corroborated by the results from the integrated analysis comprising all patients treated in the clinical development which showed a marked and durable decrease in rate of severe infections and respectively moderate and severe bleeding events.

The rate of severe infections decreased from 2.001 (95% CI: 1.5033-2.6110) infections per PYO in the 12 months period before GT to 0.154 (95% CI: 0.0418-0.3931) infections per PYO in the 6–18 month. The rate of severe infections was 0.046 (2/20 patients) at Year 5. The number of participants with severe infections decreased from 19/27 in the 12 months pre-GT to 3/26 at Y2 and 1/20 at Y5.

The rate of severe bleeding events decreased from 0.889 (95% CI: 0.5698-1.3233) events per PYO in the 12 months before GT to 0.076 (95% CI:0.0092-0.2742) events per PYO in the 12 months post GT. The rate of severe bleeding was 0.023 (1/20 patients) at Year 5 and 0 in the 1-2 years following Waskyra treatment, and the rate of moderate bleeding events decreased from 1.112 (95% CI: 0.7501-1.5870) events per PYO in the 12 months before GT to 0.721 (95% CI: 0.4341-1.1260) events per PYO in the 12 months and 0 in the 1-2 years following treatment.

An overall 96% survival rate (95% CI: 82-99%) was observed following treatment with Waskyra.

In the 26/27 surviving patients, the duration of follow-up was 24 months for 10/10 subjects and 5 years for 5/10 subjects in the pivotal study. The integrated analysis of efficacy was based on a duration of follow-up of 5 years for 20 subjects and up to 13 years for 1 subject. At the most recent data cut off (17 Jan 2025), the median follow-up of 6.816 years (range 2.31 to 13.26 years). This was considered relevant for a durable clinical benefit allowing efficacy conclusions on a comprehensive dataset.

3.3. Uncertainties and limitations about favourable effects

At the time of the data cut-off for the integrated efficacy and safety analyses (17-Jan 2025), 18 participants with severe WAS had been treated with Waskyra in the clinical studies and 9 had been treated in the EAP. The number of patients exposed to Waskyra is small, however considering the rarity of the disease, the treated population represents 20-25% of the entire EU population living with WAS, and about 50% of the Waskyra eligible population as about half of the patients would not have HLA matched donors.

Failure of engraftment remains a potentially important risk after Waskyra infusion. In case of persisting neutropenia, G-CSF should be started to stimulate bone marrow recovery based on medical judgement. If

failure of engraftment and neutropenia persist despite the use of granulocyte colony stimulating factor, administration of a non-transduced back up autologous stem cells is recommended.

As expected, given the shortened life expectancy due the disease severity, very few patients (2) were adults. A differential response between adult patients and the younger population cannot be strictly excluded given the expected long history of immune suppression in these patients. The limited data in adult patients is addressed in the SmPC.

Available data from evaluable patients indicated that the efficacy in the >5y age is at least as good as in patients aged <5 years. However, the risk of autoimmunity pre-gene therapy (GT), as well as the risk of incomplete reversal of clinical autoimmunity after gene therapy appeared higher in the age group >5 years. This risk has been addressed in the SmPC section 4.4 and will be followed up in the post approval long term PASS study.

Finally, all patients studied in clinical development were male. Although extremely rare, it cannot be excluded that a woman could be affected by WAS. Despite the lack of data in female subjects, it is not justified to exclude this population from the indication since no scientific rationale for a gender dependent clinical outcome and no differential safety would be expected.

3.4. Unfavourable effects

From the integrated safety data analysis (data cut off 17 Jan 2025), unfavourable effects reported in the clinical development program were considered as related to the protocol procedures (including $CD34^+$ mobilization, leukapheresis, conditioning or pre-treatment with rituximab, and other study procedures (the majority being related to the central venous catheter), and to the underlying disease, and/or other medical conditions.

The first 6 months after conditioning are considered particularly at risk for patients to develop severe infections, considering their increased vulnerability to pathogens, due to the ongoing of immune reconstitution. The SOC with the greatest number of AEs and SAEs was "*Infections and infestations*," which is considered consistent with the expected AE profile in this population, particularly in the early post-treatment period. Infections and infestations were reported in 44.4% of patients within the 0-6 months after Waskyra administration. It was considered related to the mobilisation, leukapheresis or preconditioning procedures prior Waskyra administration.

Overall, within this period (0-6 months after administration), among the 30 most commonly adverse events reported, petechiae (48.1%), upper respiratory tract infection (25,9%), and pyrexia (29.6%) were observed. Most of the severe of events observed were within the first 6 months post Waskyra administration (10 out of the 12 Grade 4 adverse events). These events were mainly attributable to the reduced intensity conditioning or considered related to the background disease.

One death was reported approximately 4.5 months after Waskyra infusion due to a deterioration of a pre-existing neurological condition, without any causal relationship to the study drug.

No participant developed a detectable antibody response to WASP.

Results of replication competent lentivirus testing were negative for all batches of Waskyra. No evidence of abnormal clonal proliferation was observed and none of the participants developed malignancies.

Considering the risk associated with previous HSCT and risk of immune reactions, Waskyra is contraindicated in patients with previous treatment with haematopoietic stem cell gene therapy or with allogeneic HSC transplant with evidence of residual cells of donor origin.

Overall, the safety profile of Waskyra appeared acceptable. It was well tolerated, with no AEs or SAEs related to the product observed in the clinical development program, in the short-term, as well as throughout the post-treatment follow-up period. Moreover, the safety data were consistent with expectations in patients with WAS who have undergone reduced intensity conditioning and subsequent haematological reconstitution following autologous HSCT.

3.5. Uncertainties and limitations about unfavourable effects

At the date of cut-off (17 January 2025), updated safety data showed that all 26 surviving patients completed at minimum 2 years follow up. Twenty-four (24) participants completed the year 3 visit, 20 patients completed the year 5 visit and 8 participants completed a year 8 visit.

These results remained insufficient to substantiate the long-term safety profile of Waskyra, in particular the occurrence of potential malignancies. This is expected to be further substantiated through the planned follow-up registry study (PASS) of 15 years required for gene therapy products using lentiviral vectors.

Risk of insertional oncogenesis

Based on the type of gene therapy (integrative vector), there is a theoretical risk of insertional oncogenesis that could lead to leukaemia or lymphoma after treatment with Waskyra. In the event of malignancy is suspected or confirmed, the marketing authorisation holder must be contacted for detailed instructions on sample collection for vector integration-site analysis and clonal proliferation testing. This has been addressed in the RMP and through educational material, safety tools and career /patient career card.

3.6. Effects Table

Table 22. Effects Table for [Waskyra – treatment of patients aged 6 months and older with Wiskott-Aldrich Syndrome] (data cut-off: 17 Jan 2025).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
			Waskyra N=10	12 months pre-treatment		
Rate of severe infection 6-18 months	Reduction	Number of infections per persons year of observation	0.206(0.02-0.74)	2.403 (1.54-3.57)	descriptive	OTL-103-4
Rate of moderate and severe bleeding 12 months	Reduction	Number of events per persons year of observation	0.30 (0.06-0.88)	0.901 (0.41-1.71)	descriptive	OTL-103-4

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Unfavourable Effects						
Most frequently reported adverse events in the 0-6 months post-treatment	Device related infections (33.1%)	n	27	NA	These AEs are consistent with known AEs associated with busulfan conditioning and the background disease	Integrated Safety data
Most frequently reported adverse events in the 0-6 months post-treatment	Upper respiratory tract infection (25,9%)	n	27	NA	These AEs are consistent with known AEs associated with busulfan conditioning (especially infections) and the background disease	Integrated Safety data

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The clinical package consisted of data from 27 patients treated with Waskyra in 2 clinical trials and in the EAP program, with a duration of follow-up of 5 years for 20 patients, 8 years for 8 patients and 13 years for one patient. The results of the co-primary endpoints in the pivotal clinical study and the integrated analysis showed a considerable decrease in rate of severe infections from 12 months pre-GT to 6-18 months post-GT and respectively in the rate of moderate and severe bleeding events from 12 months pre-GT to 12 months post-GT. These results are considered highly clinically relevant.

The results of the secondary endpoints: overall survival, engraftment rate, decrease in eczema severity, in rate of platelet infusions and hospitalizations were all supportive of the clinical effect.

Overall, the primary and secondary endpoints taken together demonstrated efficacy to a very high extent in term of magnitude and duration. This observed effect is also supported by the mechanism of action of the gene therapy.

Although the dataset is limited in size, it is considered proportionate to the rarity of the WAS disease. The study population is representative of the target patient population, supporting external validity, since as clarified by the applicant, the number of patients included in clinical development (27) represents 20-25% of the entire EU population living with WAS, and about 50% of the Waskyra eligible population as about half of the patients would not have HLA matched donors.

The clinical effect is sufficiently compelling in terms of magnitude (96% of patients surviving in a devastating disease with no curative option) and reliability (as reflected by the homogeneous response through the entire study population). Further the durability of the therapeutic effect was based on data from 20 patients with 5-year follow up, 8 patients with 8-year follow up and one patient with a follow up of 13 years.

This therapeutic effect has to be seen in the context of a rather well understood monogenetic disease and a simple gene replacement therapy with a well understood vector system and autologous SCT.

From the integrated safety data analysis (data cut off 17 Jan 2025), unfavourable effects reported in the clinical development program were considered as related to the protocol procedures (including $CD34^+$ mobilization, leukapheresis, conditioning or pre-treatment with rituximab, and other study procedures (the

majority being related to the central venous catheter), and to the underlying disease, and/or other medical conditions.

No signal towards specific safety issues with Waskyra were observed outside those related to the mobilisation/conditioning and administration site procedures**.**

Due to a mechanism of autoregulation of the expression of WAS in the haematopoietic stem cells, the risk of WAS overexpression is considered unlikely as investigated by the applicant. In addition, experience with other approved products with similar construct (autologous modified CD34+ cells associated with lentivirus) further inform the safety profile.

Waskyra is administered following peripheral blood mobilisation, apheresis and reduced preconditioning. The inherent associated risks are well described in the SmPC. Appropriate monitoring and precautions have been inserted in the SmPC. Further, educational materials, safety tools for health care professionals, patient careers have been defined.

The theoretical risk of malignancy due to insertional mutagenicity and the long-term engraftment will be further monitored with the requirement of 15 years follow up for treated patients, as recommended by the current Gene Therapy guideline, through the agreed long term follow up study (Cat 1 PASS study).

3.7.2. Balance of benefits and risks

Wiskott-Aldrich Syndrome is a life-threatening illness associated with a severely reduced life expectancy and median survival of 14.5 years without definitive intervention. Thus, there is an unmet medical need for an effective treatment in patients with WAS, associated with reduced life especially in case of absence of HSCT donor.

Waskyra provides the opportunity to address this unmet medical need. This is demonstrated by the highly clinically relevant effect observed and its magnitude as demonstrated by the 96% of surviving patients. The observed adverse effects were related to mobilisation procedures, preconditioning therapies and device related infections. The safety profile although limited to 27 patients, is considered acceptable. Long term safety will be monitored up to 15 years post approval as required for gene therapies.

In conclusion, the overall benefit/risk balance is positive in patients aged 6 months and older who have a mutation in the WAS gene and for whom no suitable HLA-matched related HSCT donor is available.

The recommended dose is $7.0–30.9\times10^6$ CD34+ cells per kg in one single i.v. administration.

3.7.3. Additional considerations on the benefit-risk balance

The CAT/CHMP were informed of the third-party intervention duly taken into account.

In addition, the applicant has applied for a standard marketing authorisation.

The CAT/CHMP agreed that the data package could be seen as comprehensive and supportive of a standard marketing authorisation. Indeed, in the context of the rarity of disease with well characterized natural history, the clinical effect based on both hard clinical and mechanistic endpoints, was considered sufficiently compelling in terms of magnitude, persistence, and reproducibility to overcome the limited sample size. In addition, experience with other approved products with similar construct (autologous modified CD34+ cells associated with lentivirus) further inform the safety profile. Therefore, the submitted data support a standard marketing authorisation.

3.8. Conclusions

The overall benefit/risk balance of Waskyra is positive, subject to the conditions stated in section 'Recommendations'.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described.

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 Waskyra is favourable in the following indication:

Waskyra is indicated for the treatment of patients aged 6 months and older with Wiskott-Aldrich Syndrome (WAS) who have a mutation in the WAS gene for whom haematopoietic stem cell (HSC) transplantation is appropriate and no suitable human leukocyte antigen (HLA)-matched related haematopoietic stem cell donor is available.

The CAT 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).

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 Waskyra in the treatment of patients aged 6 months and older with Wiskott-Aldrich Syndrome (WAS) who have a mutation in the WAS gene for whom haematopoietic stem cell (HSC) transplantation is appropriate and no suitable human leukocyte antigen (HLA)-matched related haematopoietic stem cell donor is available is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

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.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.
- Additional risk minimisation measures

Prior to launch of Waskyra in each Member State, the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational materials for parents/carers and health professionals, restricted prescription details and controlled access/product consent form, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The educational/ safety advice tools for health professionals should highlight the important risks of Waskyra to healthcare professionals with guidance on how to minimise the risks, including the potential risk of leukaemia/lymphoma or solid organ malignancies and the need for monitoring treated patients for signs and symptoms of oncogenic transformation, leukaemia or lymphoma or solid organ malignancies and the potential risks of engraftment failure and make them aware of the importance of monitoring and long-term follow-up,

With regards to parents/carers the educational material indicates what actions to take and when and how to contact their specialist doctor in case of side effects, any symptoms or concerns and how to report adverse drug reactions and the importance of regular and long-term monitoring.

The MAH shall ensure that, in each Member State where Waskyra is marketed, a system aimed to control its distribution beyond the level of control ensured by routine risk minimisation measures is implemented.

The following requirements need to be fulfilled before the product is prescribed, manufactured, dispensed and used:

- Waskyra will only be available through treatment centres qualified by the MAH to ensure traceability of the patient's cells and manufactured drug product between the treating hospital and manufacturing site.
- Waskyra will be administered at a specialist transplant centre, and by physicians with previous experience in the treatment and management of patients with Wiskott-Aldrich Syndrome and the use of autologous CD34+ ex vivo gene therapy products.
- A completed product consent form is required prior to initiating treatment.
- The selection of the treatment centres will be conducted in collaboration with national health authorities as appropriate.
- The healthcare professionals will receive training on the physician educational/ safety advice tools as part of the centre qualification process.

Obligation to conduct post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
Interventional post-authorisation safety study (PASS): In order to further characterise the long-term safety and efficacy of Waskyra in patients aged 6 months and older with Wiskott-Aldrich Syndrome (WAS) who have a mutation in the WAS gene for whom haematopoietic stem cell transplantation is appropriate and no suitable human leukocyte antigen-matched related haematopoietic stem cell donor is available, the MAH shall conduct and submit the results of an interventional post-authorisation study, according to an agreed protocol.	Final CSR: 31 December 2046

The CHMP endorses the CAT conclusion on the obligation to conduct post-authorisation measures as described above.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the review of available data on the active substance, the CAT considers that genetically modified autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced *ex vivo* using a lentiviral vector encoding the human Wiskott-Aldrich Syndrome (WAS) gene is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.
The CHMP endorses the CAT conclusion on the new active substance status claim

Paediatric Data

Furthermore, the CAT reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0083/2016 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

The CHMP endorses the CAT conclusion on the paediatric data.