EU Risk Management Plan
for
Waskyra (ETUVETIDIGENE AUTOTEMCEL)

A cluster of differentiation (CD)34+ cell enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced ex vivo using a lentiviral vector (LVV) encoding the human Wiskott-Aldrich syndrome (WAS) complementary deoxyribonucleic acid (cDNA).

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QPPV name:

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TABLE OF CONTENT

List of Tables

List of Abbreviations

AE	Adverse event
ANC	Absolute neutrophil count
ATG	Antithymocyte globulin
AUC	Area under the curve
BM	Bone marrow
CALD	cerebral adrenoleukodystrophy
CD	cluster of differentiation
CDMO	Contract Development and Manufacturing Organisation
cDNA	complementary deoxyribonucleic acid
CFUs	colony forming units
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
CNS	central nervous system
COI ID	Chain of Identity ID
CSR	Clinical Study Report
CUP	Compassionate Use Programme
CVC	central venous catheter
DIN	Donor ID
DMSO	Dimethylsulfoxide
DNA	deoxyribonucleic acid
DP	drug product
EAP	Expanded Access Programme
EBV	Epstein-Barr virus
EMA	European Medicines Agency
EU	European Union
EVA	ethylene vinyl acetate
FACT	Foundation for the Accreditation of Cellular Therapy
G-CSF	Granulocyte-colony stimulating factor
GFP	green fluorescent protein
gRV	Gammaretroviral

GT	Gene therapy
GvHD	Graft-versus-host disease
GVP	Good pharmacovigilance practice
HBV	Hepatitis B virus
HCPs	Healthcare professionals
HCV	Hepatitis C virus
HE	Hospital Exemption
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSC	hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
HSPC	Hematopoietic stem and progenitor cell
HTLV	Human T-cell leukaemia virus
ISA	insertion site analysis
ITT	intent-to-treat
IV	Intravenous
LTR	long terminal repeat
LVV	Lentiviral vector
JACIE	Joint Accreditation Committee - International Society for Cellular Therapy and European Society for Blood and Marrow Transplantation
MAC	Myeloablative conditioning
MAH	marketing authorisation holder
MDS	myelodysplastic syndrome
MLD	metachromatic leukodystrophy
mPB	mobilised peripheral blood
MRD	Matched related donor
mRNA	messenger ribonucleic acid
MSD	Matched sibling donor
MUD	Matched unrelated donor
NAT	Nucleic acid test
QTC	qualified treatment centre
PASS	post-authorisation safety study

PB	Peripheral blood
PCR	Polymerase chain reaction
PK	Pharmacokinetic
PL	Package Leaflet
PSUR	Periodic Safety Update Report
PT	Preferred term
RCL	Replication-competent lentivirus
RIC	Reduced intensity conditioning
RMP	Risk Management Plan
SAE	Serious adverse event
SCD	sickle cell disease
SIN	self-inactivating
SmPC	Summary of Product Characteristics
SR-TIGET	San Raffaele Telethon Institute for Gene Therapy
SUSAR	Suspected Unexpected Serious Adverse Reaction
TPO	Thrombopoietin
VCN	vector copy number
VISA	vector insertion site analysis
VITA	vector integration tag analysis
WAS	Wiskott-Aldrich syndrome
WASP	Wiskott-Aldrich syndrome protein
WIP	WASP-interacting protein
WKO	WAS knock-out
WPRE	woodchuck hepatitis virus posttranscriptional regulatory element
WT	wild-type
XLT	X-linked thrombocytopenia

PART I: PRODUCT OVERVIEW

Table 1: Product Overview

Active substance(s) **(INN or common name)**	A 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. . Telethon003 (Etuvetidigene autotemcel)
Pharmacotherapeutic group(s) (ATC Code)	Not yet assigned
Marketing Authorisation Applicant	Fondazione Telethon ETS
Medicinal products to which this RMP refers	1
Invented name(s) in the European Economic Area (EEA)	Waskyra
Marketing authorisation procedure	Centralised
Brief description of the product	**Chemical class**: Not applicable
	Summary of mode of action: Waskyra (referred to also as 'Telethon003' within the body of this document) is an autologous $CD34^+$ cell enriched population that contains HSPC transduced *ex vivo* using a LVV encoding the human WAS cDNA sequence under the control of an endogenous WAS gene promoter and a modified version of the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). Autologous $CD34^+$ HSPCs are harvested from patient mobilised peripheral blood (mPB) and transduced with a LVV, which inserts 1 or more copies of the human WAS cDNA into the cell's genome. Genetically modified cells become capable of expressing the functional WAS protein. Following administration, the genetically modified cells engraft in the bone marrow (BM) and are able to repopulate the haematopoietic compartment expressing a functioning WAS protein.
	Important information about its composition Waskyra Dispersion for Infusion is presented as a cryopreserved drug product (DP) supplied in a 50 mL (nominal volume) ethylene vinyl acetate (EVA) infusion bag (or multiple bags) at a concentration of 2-10 x 10^6 viable cells per mL, in a volume of 10 to 20 mL of cryopreservation medium ([redacted]% dimethylsulfoxide [DMSO], [redacted]% HSA and [redacted]% saline solution) per EVA bag.

Hyperlink to the Product Information	Not applicable
Indication(s) in the EEA	**Current** 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 for whom no suitable human leukocyte antigen (HLA)-matched related stem cell donor is available.
	Proposed (if applicable): Not applicable
Dosage in the EEA	**Current** The dose of Waskyra to be administered is defined based on the patient's body weight at the time of infusion. The minimum recommended dose of Waskyra is 7×10^6 CD34+ cells/kg. In clinical studies, doses up to [redacted] $\times 10^6$ CD34+ cells/kg have been administered. Waskyra is for autologous use, administered via intravenous (IV) infusion; it should only be administered once.
	Proposed (if applicable): Not applicable
Pharmaceutical form(s) and strengths	**Current** A cloudy to clear, colourless, dispersion of cells. Each patient specific infusion bag of Waskyra contains Etuvetidigene autotemcel at a batch-dependent concentration of genetically modified autologous CD34+ cell enriched population. The medicinal product is packaged in one or more infusion bags containing a dispersion for infusion of 2×10^6 to 10×10^6 cells/mL of viable CD34+ enriched cell population suspended in a cryopreservative solution.
	Proposed (if applicable) Not applicable
Is/will the product be subject to additional monitoring in the EU?	Yes

PART II: SAFETY SPECIFICATION

Part II: Module SI - Epidemiology of the indication and target population

Indication

Treatment of patients aged 6 months and older with Wiskott-Aldrich syndrome (WAS) who have a mutation in the *WAS* gene and for whom no suitable HLA-matched related hematopoietic stem cell donor is available.

Wiskott-Aldrich syndrome is an X-linked primary immune deficiency and platelet disorder (therefore primarily affecting males) characterised by microthrombocytopenia and associated bleeding, eczema, recurrent infections, and increased susceptibility to develop autoimmunity and lymphoreticular malignancies (lymphomas, leukaemias and myelodysplasias) (Ochs, 2006; Dupuis-Girod, 2003). WAS is a life-threatening illness associated with a severely reduced life expectancy, with a median survival of 14.5 years without definitive intervention (Dupuis-Girod, 2003). The main causes of death are severe infections, haemorrhages, and malignancies (44%, 23%, and 26% of cases, respectively).

WAS is caused by mutations in a single gene, the WAS gene, which codes for the Wiskott-Aldrich syndrome protein (WASP). The WAS gene is located on chromosome Xp11.23 and spans more than 9 kb. It contains 12 exons that are transcribed into a 1.8 kb messenger ribonucleic acid (mRNA) product which encodes the WAS protein. WASP is a hematopoietic-specific member of a family of cytoskeletal regulators and is found in the cytoplasm of all thrombocytes and leukocytes where it acts as an important regulator of the actin cytoskeleton, 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 and function of T lymphocytes, impaired antibody production (especially to polysaccharide antigens), defective natural killer and B cell function, reduced chemotaxis of phagocytes and dendritic cells, and functional defects of regulatory T cells. In addition, the lack of functional WASP in platelets is responsible for their small size and structural abnormalities, ultimately leading to their inability to clot blood and increased clearance from circulation.

Over 300 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 [Jin, 2004; Zhu, 1997; Notarangelo, 2003; Imai, 2004; Vallée, 2024]. Patients with mutations resulting in low residual expression of mutated protein (in most instances, missense mutations in exons 1–3) have generally been considered to be affected by the mild phenotype of WAS, also referred as X-linked thrombocytopenia; whereas those patients with mutations that prevent lymphocyte WASP expression, or allow expression of truncated WASP (e.g., nonsense and splice site mutations, deletions, and insertions) have been considered more likely to have the severe WAS phenotype. However, recent literature has underlined the high probability of developing severe complications over time in all patients with WAS, including those carrying mutations previously considered as mild [Vallée, 2024].

Despite the classical association between certain types of mutations and mild or severe phenotypes, there is no absolute genotype-phenotype association, and importantly patients initially presenting with a mild phenotype can progress to a severe phenotype and present life-threatening bleeding or severe infection, or autoimmunity and malignancies [Vallée, 2024]. Although there is a standard classification into different clinical phenotypes, WAS represents a

continuum of dysfunction due to various degrees of WASP deficiency/loss of function, and the underlying disease pathophysiology is common for all phenotypic forms of WAS.
Clinically there are 3 main features in WAS:

- Bleeding episodes: due to morphological abnormalities and reduction in number of platelets as a consequence of WAS mutation. The average incidence of bleeding manifestations before diagnosis is greater than 80% (Ochs, 2009). A significant percentage of patients (30%) present with severe bleeding episodes, the most common being haematemesis and melena. Bleeding episodes affect all WAS patients (with mild or severe phenotype).
- Infections: patients with WAS have a high susceptibility to infection from several microorganisms, including upper airway infections, invasive infections (meningitis, sepsis), viral infections (herpes simplex viruses, cytomegalovirus), fungal infections (most frequently Candida and Aspergillus) and opportunistic infections (such as pulmonary infections due to *Pneumocystis jirovecii*) (Imai, 2004).
- Eczema: this affects 80% of WAS patients during the disease and the typical skin lesions resemble acute or chronic eczema in appearance and distribution (Imai, 2004).

The severity of WAS is usually assessed using the Zhu-score system (Zhu, 1997; Ochs, 2006; Ochs, 2009), which is based on the presence of thrombocytopenia, eczema, immunodeficiency, infections, autoimmunity, and/or malignancies. The score in Table 2 is based on Zhu et al (Zhu, 1997) with subsequent refinements (Bosticardo, 2009). The milder form of WAS (XLT) is characterized mainly by thrombocytopenia and bleeding in the absence of other features, or with only mild eczema and immunodeficiency, and is scored as ≤ 2. Severe phenotypes of WAS are scored 3, 4, or 5. A score of 5A is given if there are autoimmunity manifestations and 5M if there are malignancies. Patients may move to a score of 5A or M from any severity if these morbidities are present.
The Zhu score is not static, and patients may move from one grade to another as the disease changes. This movement is invariably from a lower to a higher score, in the absence of curative treatment. Scoring before the age of 2 years may be unreliable because it often suggests a phenotype that is milder than expected from the type of *WAS* mutation identified. For this reason, scores of 1 to 2 observed in infants and young children aged less than 2 years should be re-evaluated as the patient gets older [Imai, 2004; Jin, 2004].

Table 2 Wiskott-Aldrich Syndrome Scoring System According to Zhu

	XLT			WAS			
Clinical Scores	**0.5**	**1**	**2**	**3**	**4**	**5A**	**5M**
Thrombocytopenia	+/-	+	+	+	+	+	+
Eczema	-	-	+/-	+	++	++/-	++/-
Immunodeficiency	-	-	+/-	+	++	++/-	++/-
Autoimmunity	-	-	-	-	-	+	-
Malignancy	-	-	-	-	-	-	+

WAS=Wiskott-Aldrich syndrome; XLT=X-linked thrombocytopenia
The score is based on Zhu and colleagues [Zhu, 1997], with subsequent refinements [Bosticardo, 2009].

Overall, WAS disease can present as different clinical severities ranging from a bleeding disorder alone through to patients with the classical triad of symptoms (bleeding, infections, and eczema), to patients with additional autoimmunity and/or malignancies.

Incidence and Prevalence

The overall incidence of WAS has been estimated to be between 1/100,000 and 1/1,000,000 live male births (Orphanet, 2021; Bosticardo, 2009) and the prevalence is approximately 1.9 per 1,000,000 within the population (Ceredih, 2010).

Demographics of the population in the proposed indication – age, gender, racial and/or ethnic origin and risk factors for the disease

Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency disease therefore primarily affecting males with no seeming variability in relation to race. Rare cases of females with WAS have been described involving a deleterious mutation of the paternally derived X chromosome and non-random inactivation of the maternally derived X chromosome (Buchbinder 2014). As an inherited genetic disease, there are currently no known external risk factors that might have an impact on the incidence of WAS.

The proposed indication is for the treatment of patients aged 6 months and older. In the two clinical studies, the subjects' age at the time of treatment ranged from 1.0 year to 12.4 years. However, considering the Telethon003 mechanism of action and the *WAS* genetic abnormality, pathophysiology and clinical manifestations are similar between all age groups, it is anticipated that benefit/risk profile observed in the study population could be extrapolated to all patients aged 6 months or older. Moreover, it is not expected that the safety profile of > 6-month-< 1 year-old subjects exposed to Telethon003 and to the procedure required for its administration would differ from the one reported in the age population studied during the clinical development of Telethon003.
As WAS is a progressive disease, for which the earliest possible treatment is recommended, treatment with Telethon003 is indicated from the age of 6 months. Six months is the anticipated earliest age at which gene therapy (GT) may be performed following diagnosis, including the evaluation of eligibility for GT, leukapheresis and DP manufacture. In the clinical programme there was no minimal age restriction and there is currently no minimal age limit for treatment with allogeneic hematopoietic stem cell transplantation (HSCT). Post-transplant data show 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 stem cells (Burroughs, 2020; Albert 2021). The broader age range in the indication compared with the clinical programme is supported by the comparability of efficacy and safety outcomes in patients aged < 5 and ≥ 5 years treated with Telethon003, and by data from 3 patients aged [redacted] and [redacted] years treated in the clinical development programme. Fondazione Telethon therefore proposes that Telethon003 should be indicated for patients aged 6 months and older.

The main existing treatment options:

Patients with WAS are at continuous risk of severe bleeding events, severe infections, autoimmunity, and malignancy. Therefore, the management for both prevention and treatment place a significant burden on patient's social activities and quality of life.

Current treatment options consist of conventional symptomatic and preventive management and allogeneic hematopoietic stem cell transplantation (HSCT), which can be disease stabilizing when successful.

Conservative therapy

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 (Ig) replacement therapy (IgRT), and immunosuppressive drugs, corticosteroids, and anti-cluster of differentiation (CD)20 monoclonal antibody (rituximab) for the management of autoimmune diseases.

Splenectomy

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 in addition to intrinsic platelet defects; it is less commonly used today. Moreover, this should be considered carefully when the patient is a candidate for HSCT as T- and B-cell function can be reduced in patients who undergo HSCT post-splenectomy [Moratto, 2011; Shin, 2012, Ozsahin, 2008].

Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation is used in conjunction with myeloablation and immune suppression in patients with WAS. It is mainly offered to patients with severe WAS and may be considered for milder phenotype patients (Zhu score 1 or 2) in young children with severe thrombocytopenia, severe *WAS* mutation, or absence of WASP [Mahlaoui, 2013; Albert, 2010; EBMT, 2017]. This is currently under discussion, based on recent data reporting the risk of developing disease related complications over time also for patients initially presenting with mild phenotype, supporting the need to consider definitive treatment earlier in life for these patients also [Vallée, 2024].
When successful, HSCT can lead to restoration of WASP expression, platelet count, and immune functions, and resolve eczema [Moratto, 2011; Shin, 2012; EBMT, 2017; Burroughs, 2020; Albert, 2022; Mallhi, 2021].
Complete donor chimerism (presence of >95% of donor cells in the peripheral blood [PB] and/or bone marrow [BM]) can cure the life-threatening manifestations of WAS and can be achieved using myeloablative chemotherapy [Stepensky, 2013; Stepensky, 2019]. Reduced intensity conditioning may be considered to limit drug-related toxicity of chemotherapy [Shaw, 2019]. Such a regimen is often based on use of pharmacokinetic (PK)-guided busulfan, melphalan, or treosulfan in a fludarabine-based regimen, anti-thymocyte globulin, or alemtuzumab [Lankester, 2021; Slatter, 2018; Boztug, 2016; Chiesa, 2020; Güngör, 2014]. However, reduced intensity conditioning is linked to an increased incidence of mixed chimerism, which has been associated with an increased risk of incomplete reconstitution of platelet and lymphocyte count, recurrence of clinical symptoms and post-HSCT autoimmunity [Moratto, 2011; Burroughs, 2020]. In particular, in the context of HSCT for WAS, the use of treosulfan was found to be associated with an increased incidence of graft failure, mixed chimerism, and secondary cellular therapies [Albert, 2022].

With improvements in supportive care and high-resolution human leukocyte antigen (HLA) typing methods, higher 5-year survival rates (approximately 90%) have been achieved since the early 2010s [Buchbinder, 2014; Shin, 2012; Burroughs, 2020; Albert, 2022]. Lower survival rates with HSCT were observed when patients were aged >5 years (5-year survival approximately 75%) at the time of HSCT or had severe disease as indicated by a Zhu score of 5 (5-year survival approximately 80%) [Moratto, 2011; Albert, 2022]. Hematopoietic stem cell transplantation still carries a significant risk of graft-versus host disease (GvHD), which can be debilitating and life-threatening, as well as a risk of graft failure or rejection.
Among all the possible variables influencing HSCT outcome, the two most recent surveys on patients with WAS undergoing allogeneic HSCT confirmed that age remains the most important prognostic factor, with better overall survival in patients transplanted below the age of 5 years [Burroughs, 2020; Albert, 2022; Pallas Literature Review, 2020].
The best transplantation outcome has been achieved with fully HLA-matched sibling donors and matched unrelated donors when the age of the recipient is <5 years at the time of the transplant, whereas HSCT from mismatched family donors is still associated with substantial morbidity and mortality [Burroughs, 2020; Albert, 2022].
Complications after HSCT are common, affecting about 50% of patients within the first year of treatment [Moratto, 2011; Shin, 2012], and include infections requiring hospitalization (28%), autoimmune manifestations (14–55%), graft failure or graft rejection (6–16%), acute GvHD grade >2 (12–15%), chronic GvHD (7–40%), and malignancies (3%). A higher incidence of severe complications is noticed when a mismatched family donor or umbilical cord blood donor is used [Burroughs, 2020; Albert, 2022]. Complications were observed in 60–70% of patients after HSCT from a mismatched family donor or umbilical cord blood donor, versus 47% and 25% after HSCT from a matched unrelated donors or matched sibling donor, respectively [Moratto, 2011]. The risk of complications is also greater in splenectomized patients, as well as patients with a Zhu score of 5 at the time of the HSCT, those with completely absent WASP expression, and those aged >2–5 years [Ozsahin, 2008; Moratto, 2011; Shin, 2012]. Despite the choice of 5 years as cut-off, there is evidence that the age-related risk increases linearly with age; therefore, there seems to be a consensus that HSCTs should be performed as early as possible, preferably before 2–5 years of age [Albert, 2022].
A review of the scientific literature identified 96 articles published between 2005 and 2019 reporting data on outcomes after HSCT for patients with WAS [Pallas Literature review, 2022]. Data revealed a 5-year overall survival ranging from 74% to 92% post-transplant and a 5-year event-free survival (event being defined as graft failure, graft rejection, or death) of 64% to 70%, demonstrating that although it is generally curative, HSCT still carries a risk of severe complications and death [Pallas Literature review, 2022].

Natural history of the indicated condition in the untreated population, including mortality and morbidity:

Patients with severe WAS phenotype typically present with petechiae, bloody diarrhoea, severe eczema and recurrent, often life-threatening infections; though, due to the heterogenous nature of the disease, presenting symptoms can vary at the time of diagnosis (Mallhi 2021) Patients initially diagnosed with a mild phenotype may also progress to a severe phenotype. In a series of 173 patients around 50% of patients moved from a mild to severe phenotype by the age of 30 (Albert 2010). Assessment of the severity of the phenotype before the age of 2 years may be unreliable, because it often suggests a phenotype that is milder than expected from the type of WAS mutation identified. For this reason, infants and young children with a mild presenting WAS phenotype aged less than 2 years should be re-evaluated as the patient gets older (Imai,

2004; Jin, 2004). For patients with WAS, a delay in diagnosis may be observed due to misdiagnosis including milk allergy, chronic immune thrombocytopenic purpura, idiopathic thrombocytopenic purpura, which may be due to a lack of WAS awareness (as it is a rare disease) (Albert, 2011; Yoonessi, 2015; Jin, 2019).
When treated with supportive therapy alone, patients of all WAS severities are at significant risk of disease progression and death (Buchbinder, 2014; Candotti, 2018; Pai, 2019). Data from a Worldwide WAS patient outcomes survey (n=577) by Professor Michael Albert (Albert, 2021), which forms the largest global database of WAS patient characteristics with the longest timespan, demonstrates that WAS patients who only receive supportive therapy are at considerable risk of developing serious, potentially life-threatening, events from a young age. The median time to a patient's first serious clinical event was 5 years. The proportion of patients who are event-free of any serious clinical event for this population was 33% indicating that WAS patients are at high risk of suffering from serious, life-threatening events at a young age. The most common serious clinical events were infections, followed by bleeding, autoimmunity, and malignancy. Almost all initial serious events (93.8%; n=288) occurred before 15 years of age. The infection event-free survival at 15 years was only 51% (95% Confidence Interval [CI]: 45%, 57%). The bleeding event-free survival at 15 years was 67% (95% CI: 61%, 73%). The autoimmunity event-free survival at 15 years was 70% (95% CI: 64%, 77%). Fourteen patients experienced a malignancy event before their first procedure or before their last follow-up; half of these cases (seven [50.0%]) occurred in the first 15 years of life. WAS is a life-threatening illness with a severely reduced life expectancy. Bleeding events and infections are the most frequent cause of death in patients who receive supportive therapy only (Buchbinder, 2014; Albert, 2021), and survival in a study of 55 patients with the triumvirate of symptoms was ~38% survival at 16 years and median survival of 14.5 years without definitive intervention (Dupuis-Girod, 2003).

Important co-morbidities

Autoimmunity and Autoinflammation
It has increasingly been recognised that, in contrast to autoimmunity, some immunological disorders are driven by aberration of the innate immune response; these disorders are collectively referred to as autoinflammatory diseases and these have been increasingly identified in WAS patients (Brigida, 2016; Lee, 2017; Elfeky, 2018). Other disorders, such as psoriasis, have cross-over features of both autoimmunity and autoinflammation. Autoimmune-autoinflammatory diseases are now recognised as a single group of diseases with a wide clinical spectrum, autoimmunity and autoinflammation representing each end of the spectrum (Doria, 2012). Autoimmunity is caused by a dysregulation of the adaptive immune system, resulting in the presence of autoreactive T or B cells and the production of pathogenic autoantibodies. Wiskott-Aldrich syndrome protein may be required for normal thymic maturation, and theoretically self-reactive T cells may escape negative selection because of defective T-cell receptor-induced apoptosis, as described for WASP-deficient murine lymphocytes. Increased plasma levels of B cell-activating factor and a decreased proportion of immature B cells in the BM correlating with an increased presence of transitional B cells in the periphery have been observed in WAS patients. This may contribute to the high susceptibility to develop autoimmune manifestations (Castiello, 2014). Functionally impaired WASP-deficient T cells, B cells, macrophages, or dendritic cells could all destabilize important mechanisms participating in the maintenance of normal tolerance. In addition, studies have demonstrated defects in the homeostasis and suppressor functions of natural regulatory T cells (Marangoni, 2007), which may at least partly account for the autoimmune disorders associated with WAS.
These complications are more frequently associated with severe WAS but can also develop in patients with otherwise milder disease. In addition, 25 to 36% of WAS patients suffer from more

than one autoimmune manifestation at the same time. The most common manifestations (80 % of cases) are autoimmune haemolytic anaemia, cutaneous vasculitis, IgA nephropathy and arthritis. Less common autoimmune manifestations in WAS patients are chronic inflammatory intestinal disease and autoimmune thrombocytopenia. Wiskott-Aldrich syndrome patients with autoimmune manifestations have a 25% risk of developing tumours, as compared to 5% for WAS patients without autoimmunity. In addition, 75% of patients developing tumours had signs of autoimmunity in earlier follow-up. Autoimmunity is associated with an increased risk of mortality. This is particularly true for autoimmune haemolytic anaemia and autoimmune thrombocytopenia.

Cancer
Together with severe haemorrhages and autoimmune manifestations, tumours are the major complication of WAS. They occur in 13–22% of the patients with a median age at onset of 9.5 years (Sullivan, 1994; Imai, 2004). This reported frequency is most probably an underestimation, since the median survival of WAS patients has been improving over the last decades, thereby increasing the risk to develop tumours. Wiskott-Aldrich syndrome-associated tumours are mainly lymphoreticular malignancies, with leukaemia, myelodysplasia and lymphoma accounting for 90% of the cases. This is most probably linked to the restricted expression and function of WASP in hematopoietic cells.
Immunodeficiency and specific immune-surveillance defects (such as impaired NK or cytotoxic T cell cytolysis) are likely to contribute to malignancies as some are associated with prolonged Epstein-Barr virus (EBV) infection. However, this is unlikely to be the full explanation, as the highest lymphoma risk (44%) may be conferred by a single splice site mutation that is otherwise associated with a mild clinical phenotype. Interestingly, there also appears to be a significant incidence of myelodysplasia in severe WAS patients. It has also been recently reported that WASP might play an important role in genome stability with a tumour suppressor role in blood cells (Yuan, 2020).

Part II: Module SII - Non-clinical part of the safety specification

Waskyra (formerly OTL-103 now Telethon003) is a gene therapy medicinal product comprised of an autologous $CD34^+$ cell enriched fraction containing HSPCs genetically modified *ex vivo* using a LVV encoding the human WAS cDNA sequence under the control of an endogenous WAS gene promoter and a modified version of the WPRE.

Although the clinical development of Telethon003 started before regulatory guidelines were finalized, the nonclinical development program is in line with the EMA guideline on human cell based medicinal products (EMEA/CHMP/410869/2006), the EMA Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006), the EMA guideline on quality, nonclinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008 Rev.1) and Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014), as well as the Food and Drug Administration Guidance for Industry: Preclinical assessment of Investigational Cellular and Gene Therapy Products (November 2013).

A variety of *in vitro* and *in vivo* studies support the efficacy and safety of Waskyra as a treatment for WAS-protein deficiency in humans (Module 2.4 Non-Clinical Overview).

Key safety findings from non-clinical studies and relevance to human usage are presented below:

Key Safety findings (from nonclinical studies)	Relevance to human usage
Pharmacokinetics	
Conventional absorption, distribution, metabolism, and excretion studies are not relevant for autologous cell-based products, biodistribution was evaluated as follows:	
Biodistribution	
The *in vivo* biodistribution of human $CD34^+$ cells transduced with clinical WAS LVV was evaluated after transplant into 3-4-day old immunodeficient *Rag2*$^{-/-}$*IL2r-γc*$^{-/-}$ mice (Module 2.6.4).	In human use, WAS LVV-transduced autologous $CD34^+$ cells are expected to engraft and distribute within the hematopoietic system as it is normal for these cells, as confirmed in the non-clinical models.
Transduced cells were shown to engraft and differentiate into cells of lymphoid and myeloid lineages, which were appropriately distributed to hematopoietic tissues of BM, blood, spleen, thymus, and liver.	As the vector integrates permanently into the genome and the cells are self-perpetuating, the gene modification is expected to last life-long.
No off-target engraftment and no vector mobilization or secondary transduction/integration into host bystander cells was observed, either in hematopoietic tissues or in nonhematopoietic tissues (brain or testis).	
Toxicity	
Acute or repeat-dose toxicity studies	
Waskyra is an autologous cell-based product intended for a single, one-time administration. Conventional acute toxicity and repeat-dose studies are therefore not applicable and were not conducted.	No evidence of toxicity has been seen *in vivo* in the non-clinical evaluation of Telethon003 at cell doses in the range or exceeding those expected to be administered to humans.
Instead, the toxicity risk of a single IV injection of WAS LVV-transduced cells was assessed in several mouse models: using human donor $CD34^+$ cells in immunodeficient mice tolerant of xenotransplants (biodistribution study, Module 2.6.4), or the murine equivalent Lin^- (lineage-negative) HSPCs in WAS knock-out (WKO) mice (biosafety studies, Module 2.6.6).	Rather, a selective advantage for transduced T and B cells compared to untransduced cells was observed and the overall health and longevity of WKO mice receiving a single transplant of WAS LVV-transduced Lin^- HSPCs was improved compared to mice transplanted with untransduced or WT cells.
No evidence of toxicity due to WAS LVV-transduced cells was observed in any model.	
Furthermore, the design of the vector, which includes a 1.6kb sequence of the endogenous WAS promoter, restricts transgene expression to hematopoietic lineage cells, where the level of WASP is further regulated by a requirement	

for co-expression of WASP-interacting protein (WIP). The risk of toxicity due to off-target expression or over-expression of WASP, is therefore low.

Developmental and reproductive toxicity

Conventional studies on fertility are not applicable and no DART 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 (Module 2.6.4).

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.

The risk of inadvertent germ line transmission associated with the administration of *ex vivo* transduced human cells is considered minimal according to international guidelines (*EMEA Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors, EMEA/273974/2005* and *ICH Considerations: General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors, October 2006*).

However, as conditioning regimens using busulfan are known to be associated with potentially irreversible infertility, patients and their parents/carers are advised about the options for cryopreservation of spermatogonial stem cells or ovarian tissue prior to application of conditioning in the Waskyra Summary of Product Characteristics (SmPC) and Package Leaflet (PL). Moreover, the Busulfan SmPC and PL recommend that women of childbearing potential and men capable of fathering a child must use reliable barrier contraception.

There was no exposure of Telethon003 during pregnancy in the clinical development programme.

The oldest age of a patient treated with Telethon003 in the clinical development programme was [redacted] years old (Table 7, Table 8, Module SIII).

Pregnancy and lactation are discussed further in Module SVII.1.1.

Lactation:

No non-clinical studies evaluated the presence of Telethon003 in milk.

There is no information regarding the presence of Telethon003 in human milk, its effects on milk production and its effects on the breastfed infants.

Pregnancy and lactation are discussed further in Module SVII.1.1.

Juvenile toxicity: Dedicated juvenile toxicity studies were not conducted. However, the biodistribution study (Module 2.6.4) was conducted transplanting neonate (3-4 days old) immunodeficient mice. No toxicity and no effects on organ development were reported in these animals.	Not applicable. No effects on organ development have been observed in nonclinical studies and Waskyra is not expected to have any effect on developing organs in humans. Therefore, no dedicated studies were considered necessary. (This approach was confirmed by the Committee for Medicinal Products for Human Use [CHMP] during scientific advice in the same situation for the related OTL-200/Libmeldy® [Atidarsagene autotemcel] program).
Genotoxicity No evidence of potentially genotoxic changes has been observed *in vitro* or *in vivo* in cells transduced with WAS LVV or their progeny. Vector integration analyses demonstrated no insertional hotspots and no clonal proliferation. WAS LVV was typical of LVVs, with a strong bias for integration into coding regions of the genome, but no preference for integration into or near proto-oncogenes or regions upstream of transcription start sites. The insertional profile was polyclonal in human $CD34^+$ cells both *in vitro* and after engraftment and selection *in vivo* in mice, demonstrating no evidence for clonal proliferation and thus a low potential risk of oncogenesis. Further evidence for a low genotoxic/mutagenic potential of LVV-transduced cells *in vivo* was provided by a comparison of Lin^- cells from tumour-prone *Cdkn2a*$^{-/-}$ mice transduced using prototypic lentiviral- or gammaretroviral (gRV)-vectors (expressing green fluorescent protein [GFP]) and transplanted in wildtype FVB mice. Whilst accelerated tumour onset and reduced survival was seen in mice given gRV-transduced cells, with a significant bias for integration in cancer-associated genes and for genes associated with cell cycle, LVV integrations showed no significant over-representation in any gene classes in the biological process or molecular function systems investigated and showed no acceleration of tumorigenesis (Montini, 2006).	The evidence of low genotoxic potential from investigations performed during the clinical development of Telethon003, which is corroborated by evidence from other related programs (e.g., OTL-200/Libmeldy), suggests that Telethon003/Waskyra is unlikely to be genotoxic in humans.
Carcinogenicity As Waskyra is an autologous cell-based GT intended for single administration,	In the clinical development programme of Telethon003 no cases of malignant clonal

conventional carcinogenicity studies were considered not relevant and were not conducted.

The potential tumorigenicity risk of transplantation of WAS LVV-transduced Lin^- cells was evaluated *in vivo* in the main biosafety studies (Module 2.6.6) in two WKO mouse models.

The first model, based on the C57BL/6 background strain, was followed-up for 12 months after transplant (16 months in two animals). There was no evidence of toxicity, and no tumours of graft origin occurred.

The second model, based on the SV129 background strain, was subject to a secondary transplant phase such that animals administered the primary transplant of transduced Lin^- cells were terminated after 4-months and bone marrow (BM) cells isolated and transplanted into secondary recipients. Secondary transplanted mice were followed up for a further 6 months to provide a cumulative follow-up of transduced cells for 10 months. There was no evidence of toxicity, and no graft-related tumours occurred in secondary recipients.

Overall, it was concluded that there was no evidence of toxicity and no enhanced tumorigenic effect from administration of WAS LVV-transduced Lin^- HSPCs to WKO mice.

expansion, malignancy, or adverse events (AEs) indicative of oncogenic transformation have been reported and there has been no evidence of aberrant clonal behaviour based on insertion site analysis (Section 2.1.5.2, Section 4.3, Module 2.7.4).

Integration site analysis performed on genomic DNA from whole peripheral blood (PB) and BM samples harvested at different time points after therapy showed a highly polyclonal pattern of vector integration with no indication of abnormal clonal expansion.

However, as Telethon003/Waskyra consists of $CD34^+$ cells transduced *ex vivo* with a LVV which integrates permanently in the host genome, malignancy due to insertional oncogenesis is considered an important potential risk (Module SVII.3.1).

One serious adverse event (SAE) of papillary thyroid cancer was reported in the clinical development of Telethon003, 5 years after GT. The results of vector copy number (VCN) analysis did not indicate presence of viral gene sequences within the thyroid tumour cells and therefore, the Investigator and Fondazione Telethon considered that this event was not related to Telethon003. The investigator however could not exclude a possible role played by conditioning regimen and possibly immune suppression.

The Waskyra SmPC and PL highlight that there is a theoretical risk of leukaemia or lymphoma after treatment with Waskyra. In the event that leukaemia or lymphoma is detected in any patient who received Waskyra, blood samples should be collected for integration site analysis (ISA).

Safety pharmacology

No relevant *in vitro* safety pharmacology studies were conducted and safety pharmacology studies in animals were considered neither feasible nor relevant to the administration of Waskyra to humans.

However, as the expression of WASP under the WAS promoter is restricted to hematopoietic

Not applicable.

Due to the *ex vivo* transduction of target HSCs, inadvertent expression in non-target cells (e.g., heart/CNS) is unlikely and even in such an event, adverse consequences are unlikely.

cell lines and is tightly regulated by and dependent upon co-expression of WIP, over-expression is prevented. Attempts to force increased expression with high VCNs in non-clinical studies were unsuccessful. Adverse effects at low copy numbers have not been seen and are unlikely. Additionally, Waskyra is not expected to produce proteins or enzymes which would be active within the central nervous system (CNS), cardiovascular, and respiratory systems.

Biodistribution studies confirmed that transduced cells did not populate non-target tissues in mice, including brain. Inadvertent expression in these tissues is therefore unlikely.

Drug interactions

No drug interaction studies were performed.

Based on the biologic attributes of Waskyra as an *ex vivo* genetically modified autologous cell therapy pharmacodynamic and pharmacokinetic (PK) drug interaction studies were considered not applicable.

No PK or pharmacodynamic drug interaction studies with Waskyra have been performed. Due to the nature of Waskyra, no PK interactions are expected.

Other toxicity-related information or data

Immunotoxicity

Immunotoxicity studies were not considered necessary.

Since Waskyra is an autologous cell-based product, transduced *ex vivo*, immune-mediated graft-versus host or host-versus-graft reactions are not anticipated.

Stable, long-term expression of the transgene product (WASP) was observed in mice reconstituted by WAS LVV-transduced Lin^- HSPCs, suggesting an absence of immune response against the transduced cells and the transgene product.

Patients are immune-suppressed at the time of treatment (both as part of their disease and as a result of chemotherapeutic preconditioning) and as Waskyra is administered on a single occasion only, the occurrence of immune-mediated toxicities is unlikely.

No immune response to the LVV or the transgene was observed in any of the 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.

Evaluation of secondary transduction/ vector mobilization

It has been shown that LVV particles can persist on the surface of transduced HSPCs and potentially cause secondary transduction in *in*

Whilst a theoretical risk of secondary transduction events remains, *in vivo* studies in

vitro culture with infection-permissive cell lines.

However, this effect was strongly inhibited in the presence of human serum. It is therefore considered unlikely to present any risk *in vivo*.

In addition, secondary transduction was evaluated *in vivo* in the biodistribution study (Module 2.6.4). 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 the host mouse cells.

Taken together, these *in vitro* and *in vivo* investigations raise no concerns for secondary transduction in the clinical setting.

animals for this and other programs have shown no evidence of such events.

An enhanced washing/purification step has been included in the manufacturing process to minimize the risk of carry-over of process-related impurities and it is therefore considered that there is no significant risk of secondary transduction in humans treated with Waskyra.

Generation of replication competent lentivirus (RCL)

Improvements of retroviral vector technology have led to the design of third generation self-inactivating (SIN) vectors in which the strong viral enhancer-promoter sequences of the long terminal repeats (LTRs) are deleted and, in the case of WAS LVV, replaced by a moderately active internal promoter to drive transgene expression. The viral genome is split among multiple packaging constructs such that any recombination would yield inactive/defective genomes and the use of minimal viral genes prevent even an unlikely RCL event from having the pathogenic features of a wild-type (WT) virus.

Detailed investigations into the presence of a low level of HIV-1 gag p24 capsid protein detected in plasma from a single mouse during the *in vivo* biodistribution study (Module 2.6.4) positively excluded the occurrence of an RCL event. Instead, the finding resulted from a rare plasmid integration of residual packaging plasmid carried over in the vector used for transduction of the $CD34^+$ cells.

This event was not associated with any pathology in the mouse, and it is expected not to represent any biological threat to patients.

The rare occurrence of bystander plasmid transfection in the course of vector transduction is considered a potential risk associated with vector production by transient transfection. However, this event was not associated with any pathology in the mouse, and it is expected not to represent any biological threat to patients.

Importantly, the full HIV virus cannot be formed from the plasmid constructs.

In addition, no evidence of RCL has been observed in clinical studies.

Conclusions on the non-clinical data

The safety concerns from non-clinical data based on whether the findings have been confirmed by clinical data (important identified risk), have not been adequately refuted by clinical data and/or are of unknown significance (important potential risk), or require further research (missing information) are summarised below.

Important identified risk (confirmed by clinical data)

- None

Important potential risks (not refuted by clinical data or which are of unknown significance)

- Malignancy due to insertional oncogenesis

Missing information

- None

Part II: Module SIII - Clinical trial exposure

During the clinical development programme of Telethon003 (previously OTL-103):

- **Eight subjects** were treated with the 'fresh' formulation of Telethon003 between June 2010 and September 2015 in a Phase I/II clinical study (201228).
- An Expanded Access Programme (EAP) was conducted using fresh Telethon003 following the completion of enrolment of study 201228 and prior to the initiation of study OTL-103-4. This included a Hospital Exemption (HE) program (205030) and a Compassionate Use Program (CUP) (206257. **Three patients** were treated with the 'fresh' formulation of Telethon003 in the HE programme between March and September 2016, and **six patients** in the CUP between April 2017 and January 2019. One of the nine participants enrolled in the EAP died due to an SAE that occurred in the first 6 months after Telethon003 infusion. Patient [redacted], a [redacted]-year-old [redacted] male who had been diagnosed with WAS at the age of [redacted] years, died approximately 4.5 months after Telethon003 infusion due to a deterioration of a pre-existing neurological condition (SAE preferred term: [redacted]; verbatim term: [redacted]). The patient had a medical history of spastic gait that was ongoing at screening, and a Grade 3 AE of [redacted] (verbatim term: [redacted]) with onset on Day -185. This patient's death was not considered to be related to Telethon003 by the treating physician or the Sponsor; however, relatedness to the conditioning regimen could not be excluded.
- **Ten subjects** were treated with the cryopreserved formulation of Telethon003 in a further study (OTL-103-4) between April 2019 and 30 October 2022.

In addition to the clinical trials, a reimbursed Early Access Scheme is currently active in Italy under national Law 648/96 whereby EU patients may receive Telethon 003. Patients treated under the Law 648/96 receive the cryopreserved drug product. While data from these subjects are not formally integrated into the clinical trial database, they will contribute supportive data to the overall program. As of 31 August 2024, **five patients** have been treated with cryopreserved drug product, under national Law 648/96 as shown in Table 3.

Table 3 Summary of patients treated under Law 648/96 in Italy

Patient ID	Age at treatment	Product administration date
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Table 4 Summary of Demographic Characteristics

Demography	Study 201228 (N=8)	Study OTL-103-4 (N=10)	EAPs (N=9)
Age on Date of Gene Therapy in Years, Median (Range)	2.1 ([redacted])	1.984 [redacted]	3.841 ([redacted])
Age Group on Date of Gene Therapy, n (%)			
28 Days to < 24 Months	4 (50.0)	5 (50.0)	2 (22.2)
≥ 24 Months to 11 Years	2 (25.0)	5 (50.0)	4 (44.4)
>11 years	2 (25.0)	0	3 (33.3)
Age Group (5 Year Cut-off Categories) on Date of Gene Therapy, n (%)			
< 5 Years	5 (62.5)	8 (80.0)	5 (55.6)
≥ 5 Years	3 (37.5)	2 (20.0)	4 (44.4)
Sex, n (%) [a]			
Male	8 (100)	10 (100)	9 (100)
Female	0	0	0
Race, n (%) [a]	[redacted]		
American Indian or Alaska Native			
Asian			
Black or Africa American			
White			
Ethnicity, n (%)			
Not Hispanic or Latino			
Hispanic or Latino			
Height in cm, Median (Range) [b]	90.4 (70.0, 129.5)	79.50 (66.0, 120.0)	104.2 (75.8, 172.0)
Weight in kg, Median (Range) [b]	12.6 (8.05, 35.5)	10.950 (7.59, 24.30)	20.0 (10.50, 70.00)
Body Mass Index in kg/m^2, Median (Range) [b]	15.9 (14.45, 21.47)	16.409 (13.76, 19.28	18.4 (14.36, 24.34)
Body Surface Area in m^2, Median (Range) [b]	0.5 (0.39, 1.13)	0.501 (0.38, 0.90)	0.7 (0.46, 1.81)
Country of Residence, n (%)			
Albania	[redacted]		
Bolivia			
Canada			
Germany			
Italy			
Japan			
Lebanon			
Mauritius			
Romania			
Russian Federation			

Demography	Study 201228 (N=8)	Study OTL-103-4 (N=10)	EAPs (N=9)
Spain	[redacted]		
Trinidad and Tobago			
Turkey			
United States			
Venezuela			
Time from Diagnosis to Date of Informed Consent in Years, Median (Range)	0.7 (0.02, 1.70)	0.603 (0.24, 5.08)	1.268 (0.35, 33.18)

[a] Sex and race were summarized based on data collected at screening.

[b] Height and weight, including the calculated BMI and body surface area, were based on the last measurement collected prior to treatment phase

The median duration of follow-up for the 28 participants in the Safety population who were treated with Telethon003 was 5.67 years (range: [redacted] years;). Excluding the participant who died 0.37 years after Telethon003 infusion, all participants have been followed for at least 2.31years post-GT

Table SIII.1 Clinical Trial and Expanded Access Program Duration of Follow-Up

		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

Table SIII.2 Summary of demographic characteristics at screening/baseline or on date of gene therapy (Safety population)

	Subject Exposure (N=32)
Age	
28 Days to 23 Months	13 (40.6%)
24 Months to 11 Years	15 (46.8%)
12 Years to 18 Years	2 (6.3%)
Greater than 18 Years	2 (6.3%)
Sex	

Female	0 (0.0%)
Male	32 (100.0%)
Race	
African American/African Heritage	[redacted]
American Indian or Alaskan Native	
Asian- Central/South Asian Heritage	
Asian- Japanese Heritage	
White - Arabic/North African Heritage	
White- White/Caucasian European	

Table SIII.5: Dose
All patients received a single infusion with doses ranging from 7 to [redacted] x10^6 $CD34^+$ cells/kg.

Part II: Module SIV - Populations not studied in clinical trials

SIV.1 Exclusion criteria in pivotal clinical studies within the development programme

The important exclusion criteria from the studies 201228 and OTL-103-4 are presented below. Similar to other clinical trials, patients suffering from any other major clinical condition, including end-organ functions or any other severe disease which, in the Investigator's opinion was dangerous for the patient and prevented the good conduct of the clinical trial, according to protocol requirements were excluded from clinical trial participation.

Criteria	Reason for exclusion	Is it considered to be included as missing information?	Rationale
Subjects positive for HIV infection	Including patients with HIV may have affected the safety and efficacy assessment of Telethon003 and put patients at increased risk of opportunistic infections and/or autoimmune events. Additionally, apheresis material from an HIV positive patient would not be accepted for Waskyra manufacturing.	No	This exclusion criterion, based on the list of transmissible infectious agents reported in the European Union (EU) Cell and Tissue Directive, was established for safe manipulation and to minimise the HSCT-related complications associated with the presence of certain infections and to further minimise the risk of reversion of LVV to a replication competent lentivirus. Additionally, the Waskyra SmPC advises that a negative serology test for HIV, Hepatitis C virus (HCV) and Hepatitis B virus (HBV) is necessary to ensure acceptance of apheresis material for Waskyra manufacturing. All commercial patients should be tested for HIV-1/2 prior to mobilisation to ensure acceptance of the cellular source material for Waskyra manufacturing.
Subjects affected by neoplasias.	Insertional oncogenesis is a safety concern related to the possibility of integrating viral vectors following transduction for cell modification. Patients affected by neoplastic diseases, a feature of severe WAS, were excluded so that their inclusion would not interfere with the assessment of the safety and efficacy of Telethon003 in the clinical trials,	No	Based on what is known for other gene therapies, malignancy due to insertional oncogenesis is recognised as an important potential risk of Waskyra. In the pivotal clinical trials, no persistent expansions of clones containing LVV insertions into genes associated with oncogenesis have been observed following treatment with Telethon003.

Criteria	Reason for exclusion	Is it considered to be included as missing information?	Rationale
	particularly regarding the risk of malignancy due to insertional oncogenesis.		
Subjects with cytogenetic alterations typical of myelodysplastic syndromes/acute myeloid leukaemia (MDS/AML)	Patients with cytogenetic alterations typical of MDS/AML were excluded from the clinical trials as their inclusion may have interfered with the safety and efficacy assessment of patients treated with Telethon003.	No	Based on what is known for other gene therapies, malignancy due to insertional oncogenesis is recognised as an important potential risk of Waskyra. In the pivotal clinical trials, no persistent expansions of clones containing LVV insertions into genes associated with leukaemia or myelodysplasia have been observed following treatment with Telethon003.
Subjects who had previously undergone allogeneic HSCT and had evidence of residual cells of donor origin.	This exclusion criterion was established to minimise potential confounding factors related to the clinical outcome of any previous allogeneic HSCT, which could have an impact on the evaluation of the efficacy profile of Telethon003 and related study procedures.	No	Allogeneic HSCT is currently the only potentially curative procedure for WAS. However, it is associated with significant risks, including GvHD, rejection, and complications from intense conditioning regimens. Section 4.4 of the Waskyra SmPC reports “Caution should be taken in patients who have previously failed treatment with allogeneic HSCT or haematopoietic stem cell gene therapy as this may preclude the successful harvesting of cellular source material.” as a Special warning and precautions for use.
Previous treatment with stem cell GT.	Patients having undergone previous GT would confound evaluation of Telethon003 lentiviral GT during Telethon003 clinical studies.	No	Previous GT with residual transduced cells would have been a confounding factor in the assessment of the efficacy and safety of Waskyra.

Criteria	Reason for exclusion	Is it considered to be included as missing information?	Rationale
			Section 4.4 of the Waskyra SmPC reports “Caution should be taken in patients who have previously failed treatment with allogeneic HSCT or haematopoietic stem cell gene therapy as this may preclude the successful harvesting of cellular source material.” as a Special warning and precautions for use.

SIV.2 Limitations to detect adverse reactions in clinical trial development programmes

The clinical development programme, given the relatively small number of patients involved, is unlikely to detect certain types of adverse drug reactions such as uncommon adverse reactions or adverse reactions with a long latency. The long-term follow-up of patients up to 15 years after treatment, which is being set, should however permit the identification of adverse reactions with long latency. Inclusion of additional patients will allow further opportunity, though limited given the disease incidence, to detect adverse reactions that have a less than very common or common frequency.

SIV.3 Limitations in respect to populations typically under-represented in clinical trial development programmes

Table SIV.2: Exposure of special populations included or not in clinical trial development programmes

Type of special population	Exposure
Pregnant women	Wiskott-Aldrich syndrome is an X-linked recessive disorder that primarily affects males. No women, including pregnant and breast-feeding women, were included in the clinical development programme.
Breastfeeding women	
Female population	The safety and efficacy of Waskyra in female population have not been established. No data are available. Female patients are very occasionally expected to have a WAS disease as regards this X linked genetic disease and it is not expected that they would have a differential response to treatment than male patients.
Paediatric population	The safety and efficacy of Waskyra in patients < 6 months of age have not been established. No data are available.
Elderly :	Waskyra has not been studied in patients > 65 years of age.
Patients with relevant comorbidities:	
Patients with hepatic impairment	No patients with hepatic impairment were included in the clinical development program.
Patients with renal impairment	No patients with renal impairment were included in the clinical development program.
Patients with cardiovascular impairment	No patients with cardiovascular impairment were included in the clinical development program.
Immunocompromised patients	Wiskott-Aldrich syndrome is a Primary Immunodeficiency disease. All patients in the clinical development were immunocompromised due to their disease and further by the chemotherapy conditioning regimen when treated with Telethon003.

Type of special population	Exposure
Patients seropositive for human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV)	Waskyra has not been studied in patients with HIV-1, HIV-2, active HBV, or active HCV. Perform screening for HIV-1, HIV-2, HBV, and HCV and any other infectious agents in accordance with local guidelines before collection of cells for manufacturing. Waskyra must not be used in patients with active HBV or HCV and in patients with HIV-1, HIV-2, infection.
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 not recommended 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.
Population with relevant different ethnic origin	Most patients treated in the clinical development program were of White origin ([redacted]) with a minority of patients of Asian origin [redacted]), Black or African America ([redacted]) and American Indian or Alaska Native ([redacted]). (Table SIII4 Module SIII). There are no anticipated differences in response to treatment with regards to racial or ethnic origin.

Subpopulations carrying relevant genetic polymorphisms	An assessment of *WAS* gene mutations was performed. The mutation was classified as severe/non severe based on literature data, database information, and prediction studies. Only two patients (twin brothers) had the same mutation. **Mutations of patients in the Telethon003 clinical development program.**

Patient Number	Mutation	WAS Mutation Severity	Severity of Clinical WAS Features (Zhu Score) at baseline
[redacted]	[redacted]	Severe	3
[redacted]	[redacted]	Not known	4
[redacted]	[redacted]	Severe	4
[redacted]	[redacted]	Severe	5A
[redacted]	[redacted]	Not known	4
[redacted]	[redacted]	Severe	3
[redacted]	[redacted]	Not known	4
[redacted]	[redacted]	Not known	5
[redacted]	[redacted]	Severe	5
[redacted]	[redacted]	Severe	5
[redacted]	[redacted]	Severe	3
[redacted]	[redacted]	Severe	5A
[redacted]	[redacted]	Severe	3
[redacted]	[redacted]	Severe	3
[redacted]	[redacted]	Severe	4
[redacted]	[redacted]	Severe	3
[redacted]	[redacted]	Severe	5A
[redacted]	[redacted]	Severe	5A

Type of special population	Exposure
	[redacted] Severe 2 [redacted] Severe 5A [redacted] Severe 5A [redacted] Severe 3 [redacted] Not known 3 [redacted] Severe 3 [redacted] Severe 3 [redacted] Severe 3 [redacted] Severe 3

Part II: Module SV - Post-authorisation experience

SV.1 Post-authorisation exposure

Not applicable.

Part II: Module SVI - Additional EU requirements for the safety specification

Potential for misuse for illegal purposes

Waskyra is an autologous treatment and should only be administered once. It is subject to restricted medical prescription as the product must be administered in a qualified treatment centre (QTC) with experience in haematopoietic stem cell transplantation. Waskyra has no properties that would promote its use for abuse or misuse for illegal purposes. No potential for drug dependence or drug abuse has been noted and is expected for Waskyra in the clinical development programme.

Part II: Module SVII - Identified and potential risks

SVII.1 Identification of safety concerns in the initial RMP submission

Not applicable as this is the initial Risk Management Plan (RMP).

SVII.1.1. Risks not considered important for inclusion in the list of safety concerns in the RMP

- Use in Pregnancy and lactation

Wiskott-Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency disease which therefore affects the male population. Only rare cases of females with clinical features of WAS have been described involving a deleterious mutation of the paternally derived X chromosome and non-random inactivation of the maternally derived X chromosome. No females were exposed to Waskyra during its clinical development program.

The risk of germline transmission after direct systemic administration of GT vectors is generally considered to be low (MacLachlan, 2013; Hess, 1996). As Waskyra is an ex vivo GT, the risk of germline transmission is exceedingly low. Transduction occurs ex vivo and the cell types used for ex vivo transduction do not contain gametes. The minimal risk of inadvertent germ line transmission associated with the administration of ex vivo transduced human cells is supported in international guidelines (EMA, 2006; ICH, 2006).

The potential for bystander cell transduction of germ cells (testes) upon in vivo infusion of LVV-transduced $CD34^+$ cells was evaluated and showed that LVV remained stably integrated within cells of human origin and did not mobilise to mouse tissues, including testes, confirming a low risk of germline transmission (Module SII). Due to the lack of evidence for germline transmission and the limited distribution of Telethon003 to haematopoietic tissues, no specific reproductive or developmental toxicity studies were performed.

There is no information regarding the presence of Waskyra in human milk, its effects on milk production and its effects on the breastfed infants. It is unknown whether Waskyra is excreted in human milk.

Given the target population of WAS patients being almost exclusively male, pregnancy and lactation are not considered relevant risks, howeverbreast-feeding should be discontinued during conditioning and Waskyra administration because of the potential risks associated with conditioning.

The decision to breast-feed after Waskyra treatment should be discussed with the treating physician, taking into account the benefit of breast-feeding for the child versus any potential adverse events from Waskyra or from the underlying condition.

Should pregnancy and subsequently lactation occur post-treatment either naturally or through in vitro fertilisation, pregnancy after ovo-preservation or any other method, Fondazione Telethon will monitor the progress and outcome of such pregnancies and consider whether additional actions are warranted and feasible to further characterise or minimise this risk.

- Missing information on patient population >6 months < 1 year old

In the clinical development of Telethon003, the age of the Integrated Safety patient population at the time of GT ranged from [redacted] year to [redacted] years. Eleven subjects (39%) in the integrated safety population were included in the 28 days and 24 months of age range at time of treatment with Telethon003. Eleven subjects (39%) were between 24 months and 11 years of age and 6 subjects (21%) were older than 11 years of age.

Although none of the eleven subjects between 28 days and 24 months of age were exposed to Telethon003 in clinical trials between >6 months and < 1 year of age this missing information does not automatically constitute a safety concern.

Six months is the anticipated earliest age at which GT may be performed taking into account the time needed for diagnosis, evaluation of eligibility for GT, leukapheresis and DP manufacture. The rationale for the expanded population is that the pathophysiology and clinical manifestations of WAS are considered similar in patients aged < 1 year and > 12 years old as the subjects who participated in the clinical trial program. Additionally, Waskyra has the same mechanism of action for all patients ages.

A recent study on the treatment benefit of allogeneic HSCT for WAS enrolled 129 subjects with a median age of 1.2 (range, 0.2–21.7) years (Burroughs 2020). Fifty-three patients (41%) received HSCT <1 year of age. The 5-year survival among infants <1 year was 94%, young children 1-1.99 years 95%, and children 2 to 4.99 years 92% (Burroughs, 2020) which shows that in allogeneic HSCT age from <1 year old to 4.99 years old was not a major contributor to survival. The safety profile in this study was consistent with the one reported in the clinical development programme for Telethon003.

Good pharmacovigilance practices (GVP) Module V rev2. states that: "*The absence of data itself (e.g., exclusion of a population from clinical studies) does not automatically constitute a safety concern. Instead, the risk management planning should focus on situations that might differ from the known safety profile. A scientific rationale is needed for the inclusion of that population as missing information in the RMP*."

Considering the data presented above, it is not expected that the safety profile of >6 months < 1 year old subjects exposed to Telethon003 and to the procedure required for its administration, would differ from the one reported in the age population studied during the clinical development of Telethon003. Therefore, given the lack of a supporting scientific rationale, it is considered that the >6 months old < 1 year old population should not be included as missing information in the RMP of Telethon003.

- Immunogenicity

The risk that gene therapy derived WASP could induce unwanted immune responses in treated patients was investigated during the Telethon003 clinical development program. 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.

Immune-mediated adverse events that were identified by a preferred term search using the SMQ for immune-mediated and autoimmunity events are described in the clinical summary of safety (Section 2.1.4.1). Wiskott-Aldrich syndrome is a primary immunodeficiency and autoimmunity is a clinical feature of severe WAS.

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.

Twenty AEs were reported in 15 (55.6%) participants in the first 48 hours after Telethon003 infusion (Table 2.7.4.2.19). 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).
None of these events was classed as an SAE .
These data attest to the short-term safety and tolerability profile of the Telethon003 infusion.

Due to the lack of clinical data suggesting the induction of autoimmunity or immunogenic reactions following Waskyra infusion and in light of the confounding factors played by the conditioning and pre-treatment regimen, Fondazione Telethon does not consider that the risk of immunogenicity should be included as a safety concern in the RMP.

- Adult population

Currently available data, though limited, do not indicate differences in clinical outcome between children and adults.

There is no safety concerns regarding the drug product:

- Good engraftment and increased platelet count in both adults treated
- No observed events or concerns about ISA, insertional mutagenesis and oncogenesis, RCL, or transgene immunogenicity
- Sustained clinical improvement of the adult who was followed up for 6.2 years

Reason for not including an identified or potential risk in the list of safety concerns in the RMP

Not applicable.

Risks with minimal clinical impact on patients (in relation to the severity of the indication treated)

None.

Adverse reactions with clinical consequences, even serious, but occurring with a low frequency and considered to be acceptable in relation to the severity of the indication treated

None.

Known risks that require no further characterisation and are followed up via routine pharmacovigilance namely through signal detection and adverse reaction reporting, and for which the risk minimisation messages in the product information are adhered by prescribers (e.g., actions being part of standard clinical practice in each EU Member state where the product is authorised)

- Risks related to medical and surgical procedures (e.g., central line placement, leukapheresis)

Similar to other autologous ex vivo HSPC gene therapies, there are multiple steps involved in Waskyra administration including central line placement and leukapheresis that are recognised to be associated with potential complications such as infection and thrombosis. As part of the Waskyra administration process, a central venous catheter (CVC) is inserted under general anaesthesia for the administration of chemotherapy and IV medications and aseptic procedures should be adhered to. Other procedures include leukapheresis.

Infections are common when a CVC is in place (WHO, 2014). Patients who are immunocompromised are at increased risk of infections. Catheter occlusions and catheter-related thrombosis are known complications of catheters and catheter-related thrombosis occurs in up to 50% of children with a long-term CVC (Baskin, 2009). Central venous access is a requirement for all patients to receive a conditioning regimen and to infuse either allogeneic HSCT or Telethon003.

Rates of CVC infections vary widely based on patient population, device type, frequency of CVC manipulations, and dwell period (Fratino, 2005; Martynov, 2020). In paediatric oncology patients without HSCT, catheter-associated infections ranged from 14 to 58% (Martynov, 2020). In a prospective study of 418 CVCs placed in 368 children in 2 tertiary care centers in Italy following up 3 types of CVCs used over a 30-month period, 234 complications were recorded; the most frequent complication was infection at a 40% rate (Fratino, 2005).

In the Integrated Safety Set part of the marketing application submission files device-related infections occurred in 10/27 (37%) of subjects in the post-treatment phase

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. Such events are not unexpected following the installation of ports for central venous access, which is required to administer busulfan conditioning and Telethon003. Additionally, there was one event of device related infection in the 1–2-year period.

Therefore, the risks related to medical or surgical procedures (e.g., central line placement, leukapheresis) such as serious CVC infections and thrombosis in the device are not important risks of Waskyra per se but risks related to the required procedure / associated therapies without which Telethon003 could not be administered. These risks are well-recognised and can be managed in clinical practice through patient monitoring and standard of care treatment as described in the guidance provided in section 4.4 of the Waskyra SmPC.

In order to minimise the risks related to medical and surgical procedures, Waskyra must be administered in a QTC. The Waskyra SmPC includes guidance on the method of administration

including the precautions to be taken before handling or administering the medicinal product, and guidance on preparation for infusion, administration and after administration. Healthcare professionals (HCPs) are also advised that infections related to the use of CVCs have been reported in clinical trials and as there is a theoretical risk of thrombosis associated with the CVC, patients should be closely monitored for potential infections and catheter-related events.

The risks related to medical or surgical procedures (e.g., central line placement, leukapheresis) will continue to be monitored in clinical practice using routine pharmacovigilance.

- Risks related to mobilising agents

Mobilisation of stem cells into the PB is generally performed using Granulocyte-colony stimulating factor (G-CSF), a glycoprotein which regulates the production and release of functional neutrophils from the BM. The combination of G-CSF with plerixafor can be used to increase the responsiveness of individuals to mobilisation and increases the yield of haematopoetic stem cells (HSCs) from mobilisation. Plerixafor has been recently authorised in the EU for use in combination with G-CSF to enhance mobilisation of haematopoietic stem cells to the PB for collection and subsequent autologous transplantation in children with lymphoma or solid malignant tumours. It further minimises the need for multiple rounds of apheresis to produce enough stem cells for Waskyra manufacturing.

G-CSF was administered twice daily (total dose of 10 µg/kg to 12.5 µg/kg, administered in 2 divided doses), in order to mobilise $CD34^+$ cells from the BM into PB (Ravagnani, 1990). On the fourth and fifth day of G-CSF administration, plerixafor, was administered to enhance mobilization into the PB (Vose, 2009; Yannaki, 2013; Gardellini, 2013; Aabideen, 2011; Vettenranta, 2012). The starting day of plerixafor administration could be shifted to the fourth or fifth day depending on the white blood and $CD34^+$ cell count in the subjects' PB. Plerixafor was administered subcutaneously once daily at a dose of 0.24 mg/kg, approximately 6 - 8 hours before standard leukapheresis. Mobilizing agent administration was repeated until sufficient mobilized PB $CD34^+$ cells had been harvested, up to a maximum of 3 leukapheresis rounds on 3 consecutive days, or 7 days of G-CSF ± plerixafor administration.

During treatment with G-CSF ± plerixafor, a complete blood cell count was obtained daily and a PB $CD34^+$ cell count from the third day. Leukapheresis was performed as soon as the mobilized PB $CD34^+$ cell count reached an adequate level.

In study OTL-103-4, one subject had an AE of gastrointestinal disorder (verbatim: "gastrointestinal symptoms after plerixafor administration") that was considered related to the $CD34^+$ HSPC mobilization procedure. In the Integrated Safety population, no SAEs considered related or probably related to $CD34^+$ HSPC mobilization procedure were reported in any of the subjects.

Risks related to mobilising agents are not considered an important risk of Waskyra as they are not specifically associated with Waskyra, although the use of mobilising agents is a required part of the treatment process without which Waskyra could not be manufactured. Patients treated with G-CSF with or without plerixafor are expected to experience adverse drug reactions, but these risks are well-recognised for G-CSF and plerixafor and can be managed in clinical practice through patient monitoring and standard of care treatment as described in the SmPCs of the involved products (Filgrastim SmPC; Mozobil SmPC).

- Risks related to pre-treatment and conditioning

Before Waskyra infusion, subjects received a pre-treatment with rituximab followed by a RIC with busulfan/fludarabine.

Rituximab is an anti-CD20 monoclonal antibody which is used as a depleting agent for B cells and particularly autoreactive cells, thereby facilitating the engraftment and expansion of gene corrected B cells expressing WASP. In addition, rituximab acts as pre-emptive treatment for lymphoproliferative disorders due to EBV, which represents a high-risk factor for the development of lymphoma in WAS patients. Whereas MAC is predominantly used for allogeneic HSCT in order to obtain full chimerism, RIC was selected in the Telethon003 clinical development program as mixed chimerism does not pose any safety risk in an autologous setting.

All 27 treated subjects received rituximab and conditioning treatment. In one patient in the EAP the rituximab infusion had to be stopped because of an SAE of shock. The rituximab dose administered to this patient was only 7 mg/m^2. The other patients received a dose between 333 mg/m^2 and 387 mg/m^2. The median rituximab dose was 371 mg/m^2. Fludarabine was given at a dose of 60mg/m2 was given as a divided dose on Days -3 and -2. The target range of busulfan cumulative area under the curve (AUC) was 49,856ng·h/mL (± 10%) while the total cumulative busulfan AUC ranged from 33,314 ng·h/mL to 81,012 ng·h/mL.

Exposure to busulfan was significantly higher than the target range of cumulative AUC for two subjects. In spite of correct application of busulfan dosing and PK monitoring as per protocol. One subject reported a busulfan AUC of 63,000 ng·h/mL and one subject of 81,012 ng·h/mL. While the former did not develop any AEs following high exposure to busulfan, the latter developed 2 SAEs, venoocclusive liver disease and suspected thrombotic microangiopathy, which are both expected according to the Busilvex SmPC (Busilvex SmPC, last updated: 15 Mar 2021). In order to minimise the risk of higher than anticipated exposure the study protocol was amended to modify the busulfan PK blood sampling schedule and give the possibility not to administer the eighth (last) dose of busulfan if the targeted exposure is reached by the seventh dose.

As presented in Section 2.1.1.5.2, Module 2.7.4, a specific process was followed for the selection of adverse drug reactions potentially attributable to RIC or pre-treatment using the integrated data set. After a comprehensive assessment involving a clinical evaluation, with consideration of similar preferred terms (PTs), biological plausibility, nature and timing of the events, the underlying disease, incidence of the event in the paediatric population and a comparison with the adverse drug reactions listed in the busulfan plus fludarabine SmPC (Busilvex, updated Oct 2020), rituximab SmPC (MabThera, 20 Sep 2021), plerixafor SmPC (Mozobil, updated 01 Jan 2021), filgrastim (Neupogen Singleject, updated 03 Dec 2020) and lenograstim SmPC (Granocyte, updated Nov 2020), a total of 58 PTs from 14 SOCs were determined by Fondazione Telethon to be potentially attributable to pre-treatment or RIC.

The most frequently reported AEs possibly related to pre-treatment and conditioning reported from the start of the treatment period to 100 days after Telethon003 infusion were pyrexia and anemia, both reported in the Busilvex SmPC. Seventeen events of pyrexia were reported in nine (32.1%) participants in this period, with six events in six (21.4%) participants in the on-treatment phase and 11 events in six (21.4%) participants in the first 100 days after Telethon003 infusion. In addition, 16 events of anemia were reported in six (21.4%) participants, with 10 events in five (17.9%) participants in the on-treatment phase and six events in two (7.1%) participants in the first 100 days after Telethon003 infusion.
Across all adverse events possibly related to pre-treatment and conditioning, the most frequently reported SOC from the start of the treatment period to 100 days after Telethon003 infusion was “Gastrointestinal disorders”, with 47 events reported in 23 (82.1%) participants. Fifteen events were reported in 10 (35.7%) participants in the on-treatment phase and 32 events in 18 (64.3%) participants in the first 100 days after Telethon003.

Serious AEs that were considered related to conditioning by the Investigator/treating physician were reported in one (3.6%) participant in the on-treatment phase (shock, considered to be related to rituximab) and two (7.4%) participants in the first 6 months after Telethon003 infusion (neutropenia, thrombotic microangiopathy, and venoocclusive 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. No conditioning-related SAEs were reported beyond 6 months after Telethon003 infusion.

Two SAEs (papillary thyroid cancer and neurological decompensation) were considered as "possibly related" to the conditioning regimen.

The adverse drug reactions potentially attributed to pre-treatment and RIC are listed in section 4.8 of the Waskyra SmPC and the PL and section 4.4 of the SmPC advise that the warnings and precautions of the pre-treatment and RIC agents must be considered.

The risks related to the conditioning regimen are not considered an important risk of Waskyra as they are not specifically related to Waskyra even though a conditioning regimen is a required part of the treatment process and without RIC Waskyra may not be effective. The safety profile of busulfan plus fludarabine and are well established after years of use in transplant setting and the risks can be managed in clinical practice through patient monitoring and standard of care treatment as described in the relevant SmPCs of the involved products (Busilvex SmPC).

Known risks that do not impact the risk-benefit profile

Waskyra contains [redacted]% DMSO as an excipient. DMSO is the most commonly used cryoprotectant and is associated with a risk of dose- related toxicity. Although generally mild in nature, can also include more serious events of hypersensitivity/anaphylactic reactions (Kollerup Madsen, 2018). Section 4.4 of the Waskyra SmPC states that: "*Dimethylsulfoxide (DMSO), one of the excipients of Waskyra, is known to possibly cause anaphylactic reactions following parenteral administration. Patients not previously exposed to DMSO should be observed closely. Vital signs (blood pressure, heart rate, and oxygen saturation) and the occurrence of any symptom should be monitored prior to the start of the infusion, approximately every ten minutes during the infusion and every hour, for 3 hours, after the infusion*".

Moreover, the Waskyra SmPC and PL also advise that when more than one bag of Waskyra **is needed,** only one bag of medicinal product should be infused at a time. **It should be ensured prior to** infusion that the volume of medicinal product to be infused is compatible with the limit of DMSO recommended by the Committee for Advanced Therapies (i.e., the total volume of DMSO administered should remain < 1% of the patient's estimated plasma volume). Therefore, the maximum volume of Waskyra to be administered should remain < 20% of the patient's estimated plasma volume.

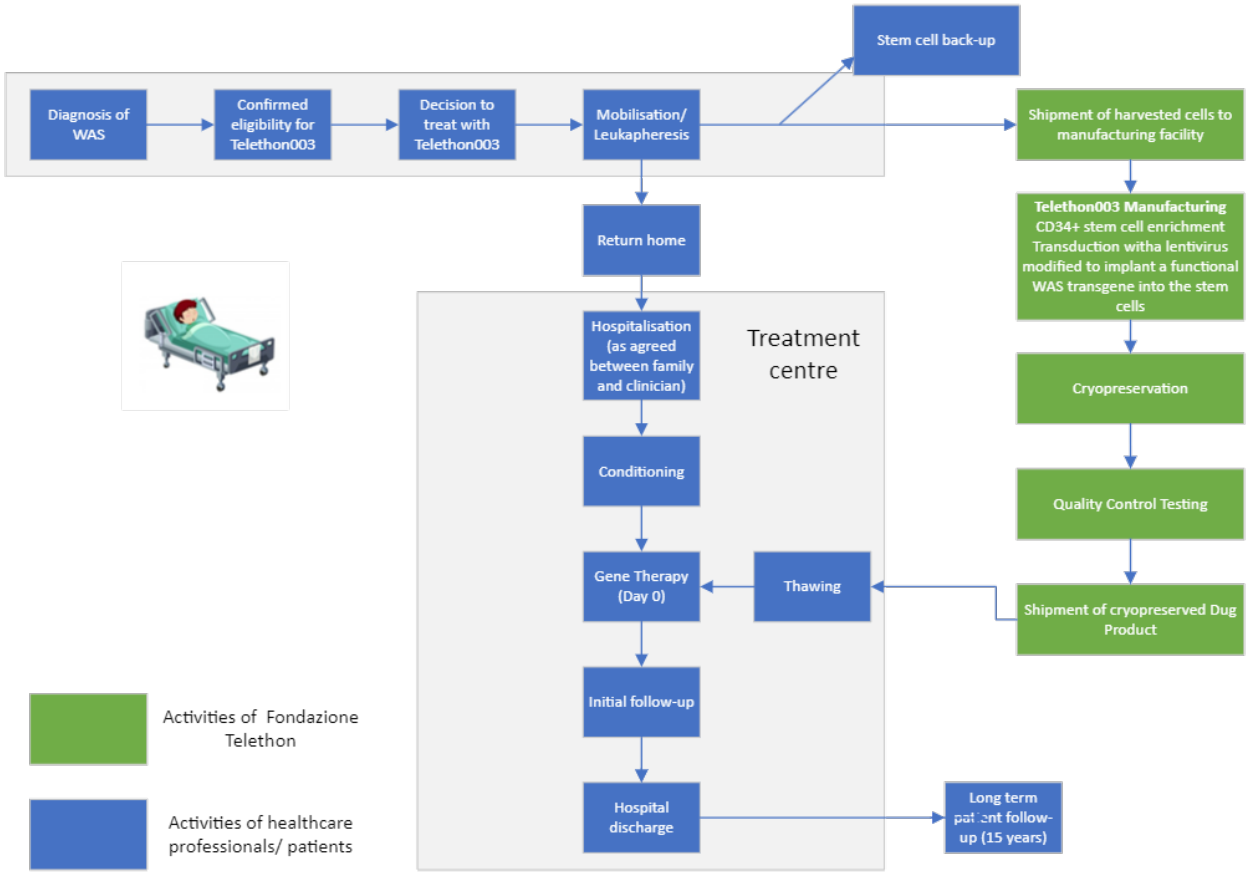
Other reasons for considering the risks not important

None.

Advanced therapy medicinal product specific risks

Waskyra is a GT that requires a multistep manufacturing process (Figure 2). Waskyra is an autologous GT product, therefore, ensuring the product is linked from autologous cell procurement back to the same patient for treatment with DP is essential.

Figure 1 Flow Chart of the Logistics of Therapy



The applicant is implementing a traceability system to ensure that Waskyra and its "starting and raw materials", including all substances that get in contact with the cells or cellular sources, can be traced through the sourcing, manufacturing, packaging, storage, transport, and delivery, as described in Module 3. The traceability system tracks collection of cellular source material, shipment to the manufacturing facility, manufacture and storage of the DP, shipment of DP back to the QTC, and receipt of DP at the QTC.

Risks to patients in relation to quality characteristics, storage, and distribution of the product

Incorrect storage and handling may pose a risk to the viability of the cells within the product and, therefore, could in theory pose a risk to patients or damage the product so that it may not be suitable for administration. Unsuitable DP may result in the need for administration of the un-manipulated back-up to reconstitute the patient's haematopoietic system following conditioning.

The Telethon003 DP is cryopreserved under the vapour phase of liquid nitrogen and stored at ≤ -130°C until the specific instructions for thawing of the product are followed prior to administration. Stability studies were performed using $CD34^+$ cells isolated from healthy donor

material (BM or mPB), transduced with ex vivo LVV encoding the human WAS gene and stored in an EVA cryobag at the recommended storage condition ≤ -130°C. Stability data support a 6-month shelf-life for cryopreserved Telethon003 DP when stored at ≤ -130°C. Once thawed, the maximum shelf-life at room temperature (20°C-25°C) is 2 hours.

Telethon003 is shipped in a validated liquid nitrogen (LN2) dry vapor shipper and will remain stable throughout shipping duration for up to 5 days. The pack-out of Telethon003 DP is strictly controlled by the Contract Development and Manufacturing Organisation (CDMO), and the receipt and handling of Telethon003 DP at the QTC is controlled by the clear instructions provided in the Product Manual. The shipment of Telethon003 is performed by trained couriers, and the process is controlled by a specialist distribution and logistics provider.

Critical characteristics of the DP are controlled at release through testing according to the specification. Distribution of the product is qualified by a mock shipment activity to qualify the shipping route between a treatment site and CDMO for shipment of cellular source material and cryopreserved DP.

The mock shipment for each specific treatment site confirms that patient cellular source material and cryopreserved DP can be transported between a treatment site and CDMO within an acceptable timeframe. The documentation is demonstrated to be sufficient for the shipment of patient material between the two sites. Mock shipments also confirm that the specified temperature range can be maintained during transport across the shipping route. Fondazione Telethon's Distribution Team will monitor and assess all future shipping activities to ensure adherence to the acceptance criteria.

It is recommended that each bag of Telethon003 is infused immediately post-thaw, however, the stability of Telethon003 permits an infusion within 2 hours post-thaw if needed. Providing the storage and administration procedures for Telethon003 are followed as per the SmPC guidance, this does not represent a safety concern.

Administration

Waskyra must be administered in a QTC with experience in HSCT, by a physician with previous experience in the treatment and management of patients with WAS and in the use of autologous $CD34^+$ ex vivo GT products or HSCT. Waskyra should only be administered after consultation and signature of the Informed Consent Form with the patient and/or family.

Patients who receive the Waskyra will be invited to participate in a Long-Term Follow Up study. Waskyra is intended for autologous use only (see Warning and Precautions in Section 6.3 of the SmPC).

Before infusion, it must be confirmed that the patient's identity matches the essential unique patient information on the Waskyra infusion bag(s) and container. The contents of each cryopreserved bag should be fully administered within 2 hours of being thawed.

Patients should be monitored closely prior to, during, and after infusion. Vital signs (blood pressure, heart rate, and oxygen saturation) and the occurrence of any symptom should be checked every ten minutes during the infusion and every hour, for 3 hours, after the infusion

Patients should be monitored frequently by complete blood count for at least 6 weeks after infusion or until recovery of hematopoiesis and infections managed according to standard guidelines and medical judgement.

Table 5 Preparation/Handling/Storage/Accountability

	Telethon003 (cryopreserved/commercial formulation)
Shelf-life	Expiry date and time are stated on the product label. Stability studies currently support a six-month shelf-life for cryopreserved Waskyra. Each cryopreserved bag of Waskyra should be administered within two hours of being thawed.
Storage	Store at < -130°C in the vapor phase of liquid nitrogen
Transportation	Details of the cold-chain transportation, storage and thawing of Waskyra will be detailed within the Product Manual

Risk of transmission of an infectious agent

Harvest of patient $CD34^+$ HSPCs from mPB is performed at the QTC. Mobilised peripheral blood harvest is conducted according to the standards and principles described by the Joint Accreditation Committee - International Society for Cellular Therapy and European Society for Blood and Marrow Transplantation (JACIE) or Foundation for the Accreditation of Cellular Therapy (FACT). The HSPC collection facilities must also be JACIE/FACT accredited and have a Tissue Establishment License as per Directive 2004/23/EC in Europe. Additionally, the sites used conform to the Directive 2004/23/EC and sister directives for donation, procurement and testing as well as traceability requirements.

The harvested $CD34^+$ HSPCs are transported to the manufacturing facility via a qualified transport provider. Chain of custody begins at the collection of HSPCs and continues through transport to the CDMO and the DP back to the QTC. The starting material is transported in a validated shipping container with a datalogger for tracking the temperature during transit. Quality Agreements are executed between the collection facility and Fondazione Telethon, and Fondazione Telethon and the transport providers.

Prior to initiation of the HSPC collection procedure from mPB, patients are tested for the presence of infectious agents as required by Directive 2004/23/EC and related directives.

All raw materials are procured from suppliers approved by CDMO under their Vendor Management Programme. Quality agreements are executed with critical raw material suppliers. Where available, material manufactured in compliance with Good Manufacturing Practices and compliant with current compendial monographs are used for manufacture of Waskyra drug substance.

The CDMO requires a declaration of compliance with EU Directive 2006/17/EC as amended from the clinical site as part of the documentation needed for the first step for release of the material prior to use, thereby assuring compliance to relevant standards of quality, safety testing, processing, preservation, storage and distribution of human cells and tissues.

To minimise the risk of transmission of an infectious agent, incoming cells are tested for infectious diseases and must be confirmed as acceptable prior to HSPC release:

- Verification of the accompanying documentation as defined in Table 5
- Microbiological examination of cell-based preparations, based on Ph. Eur. 2.6.27 guidelines with an acceptance criteria or culture negative for Microbiological control (or similar qualified method).

Table 6 Verification of Documentation for Mobilised Peripheral Blood (Autologous)

Description of Control	Acceptance Criteria
Chain of Identity ID (COI ID) and Donor ID (DIN) in documentation matches the same codes on primary packaging	Yes
Documentation states that donor is negative for HCV1, HBV, HIV1/2, HTLV1/2 (if applicable) and *Treponema pallidum* as per Directive 2006/17/EC as amended	Yes
Documentation states that donor is negative for mycoplasma	Yes
Execution date of virus screening is consistent with required timing as per Directive 2006/17/EC as amended	Yes
Confirmation that sample collection was carried out in accordance with Directive 2004/23/EC and sister directives	Yes
The primary packaging of the product is intact	Yes

Note: 1. Prior to mobilization and cell harvest a nucleic acid test (NAT) with a limit of quantification of ≤15 international units/mL must be used to confirm the absence of ongoing HCV infection, i.e., "negative for HCV". In cases where patients have previously tested positive for HCV infection, negative NAT results are required on at least 3 sequential occasions over a period of at least 4 weeks, with the final test conducted no more than 3 days prior to cell harvest.
Abbreviations: COI ID = Chain of Identity ID; DIN = Donor ID; HCV = Hepatitis C virus; HBV = Hepatitis B virus; HIV = Human immunodeficiency virus; HTLV = Human T-cell leukaemia virus; NAT = Nucleic acid test

Although Waskyra is tested for sterility and mycoplasma at the final release, because a small risk of transmission of infectious agents exists, this risk is communicated to healthcare professionals through the product information. As a precautionary measure, the Waskyra SmPC advises healthcare professionals administering Waskyra to monitor patients for signs and symptoms of infections after treatment with Waskyra and to treat appropriately, if needed.

The only other source of infectious agents would come from the vector since disposable materials for the manufacturing process are used. Lentiviral vector lots are tested for sterility, adventitious agents including mycoplasma and infectious virus, RCL and viral potency prior to release for use in the Waskyra manufacturing process.

Environmental risks

There is no risk concerning the contamination of the environment with Waskyra. Genetically modified HSPC are not deliberately released in the environment and are not able to survive outside of the human body unless they are specifically cultured in humidified temperature- and gas-controlled incubators or in an adequate live model. As patient HSPC are genetically modified ex vivo, the probability of free viral particles being associated with the cells at administration is very low. Lentiviral particles that have not entered into and transduced the HSPC are removed

during the manufacturing process and, even under cultured conditions, they have a short half-life (Merten, 2004). In addition, there are no known mechanisms to enable shedding of WAS LVV from cells transduced with Waskyra as these cells do not contain the required viral elements to mobilise the WAS LVV and produce infectious virions.

Although shedding of WAS lentiviral particles has not been assessed as part of a non-clinical programme, no shedding of the genetically modified sequences is expected.

Even in the unlikely case that some free viral particles are present, the vector is replication-deficient and thus the expansion or survival of WAS LVV in the environment is impossible as such viral particles have an extremely low fitness to survive when exposed to environmental conditions.

In conclusion, it is considered that WAS LVV particles used to modify the patients' own HSPC ex vivo are not shed by the patients into the environment via saliva, urine, or faeces.

Generation of replication competent lentivirus

The viral genome is split among multiple packaging constructs such that any recombination would yield inactive/defective genomes and the use of minimal viral genes prevent even an unlikely RCL event from having the pathogenic features of a WT virus. The incorporation of a mutated WPRE with a 6-nucleotide change (WPREmut6) abrogates a potential hepatocarcinogenic risk by preventing expression of a 60-amino acid peptide of protein X.

Bone marrow cells harvested from the mouse at sacrifice showed no evidence of RCL in culture, contained no vesicular stomatitis virus genomic DNA and were negative in a B2-SINE-PCR assay that scores for vector mobilization from human to mouse cells. These results indicated the absence of any recombinant replication-competency and confirmed that no transfer of packaging function (gag expression) had occurred.

To date there has been no reported evidence of positive RCL results in either clinical lentiviral vector lots, ex vivo lentiviral GT lots, or patients infused with these GT products (Marcucci, 2018; McGarrity, 2013; Cornetta, 2018).

There were no positive results of RCL in any of the 27 subjects treated as part of the Telethon003 clinical development program at time of data cut (Section 4.2, Module 2.7.4). Other published studies have found no evidence of RCL including an evaluation of test results of 17 clinical vector lots, 375 manufactured T cell products, and 308 infused patients from both oncology and HIV clinical trials infusing retroviral- or lentiviral-transduced T cells from a total of 194.8 post-infusion person years of RCL follow-up (Marcucci, 2018). Cumulatively, on more than 80 subjects in the marketing authorisation holder (MAH) clinical development program who have been exposed to LVV-based gene therapies, none have expressed RCL up to data lock point of this RMP.

SVII.1.2. Risks considered important for inclusion in the list of safety concerns in the RMP

Important Identified Risks

None

Important Potential Risk 1

Malignancy due to insertional oncogenesis

Malignancy due to insertional oncogenesis is considered an important potential safety concern as Waskyra consists of $CD34^+$ cells transduced ex vivo with a LVV which integrates permanently into the host genome.

The standard battery of genotoxicity tests is not considered appropriate for Waskyra, but a range of in vitro and in vivo tests are reported. Overall, it is concluded that WAS LVV presents a very low risk for mutagenesis or cancer.

An in vitro immortalization assay, based on the detection of clonal dominance of insertional mutants, was conducted to evaluate the potential genotoxicity of the WAS LVV (Modlich, 2009). No induction of re-plating activity was observed in four independent assays conducted with WAS LVV, indicating a low mutagenic potential for this vector.

Vector insertion site analysis (VISA) was performed to interrogate the integration profile of the WAS LVV (w1.6W_mut6) in healthy donor or WAS patient $CD34^+$ cells in vitro, and in cells from healthy donors in vivo after engraftment in Rag2−/−Il2rγc−/− mice (Scaramuzza, 2013).

The insertion profiles in vitro and in vivo confirmed the preference of LVV for transcriptional units inside genes and without any bias for promoter regions. The pattern of insertions was highly polyclonal both in vitro in human $CD34^+$ cells from HD and WAS patients and in vivo upon infusion in Rag2−/−Il2rγc−/− mice. No differences were observed from the use of different vector batches or with 1- or 2-hit transduction protocols.

Further evaluation of the potential genotoxicity of WAS LVV integration in vivo was conducted using a high-throughput (vector integration tag analysis [VITA]) technique to tag and identify vector DNA in the SV129 WKO mouse genome following ex vivo transduction and transplant of murine WAS-/- Lin- cells into lethally irradiated recipients (Mantovani, 2009). This study also confirmed a polyclonal pattern of integration in transcription units within genes and no hotspots of insertion.

To further explore the risk of insertional oncogenesis, primary SV129 WKO mice were transplanted with Lin- WKO BM cells transduced with the w1.6W_mut6 LVV at MOI 100 using either a 1-hit or a 2-hit transduction procedure (WA 1X and WA 2X experimental groups) (Marangoni, 2009). Transduced cells (1x105 cells/mouse) were transplanted in sex-mismatched lethally irradiated mice and followed for 4-months.

No tumours were found, and no evidence of GT-related toxicity was observed. Four months after primary graft, mice with the highest vector copy number (VCN) or Y chimerism in PB were chosen as BM donors for secondary transplants (2-3 selected from each of the 1- or 2-hit transduction procedures). Bone marrow cells were collected from each donor and $2x10^6$ cells re-transplanted into 5 or 6 secondary recipients (n=16 per group for WA 1X and WA 2X groups and n=11 for control groups). The secondary recipient mice were observed for 6 months. Engraftment, survival, blood counts, macroscopic observations and histological evaluation of tumors were assessed.

Morbidity and mortality were similar between treated and control groups, with deaths attributed to the irradiation conditioning and underlying WKO-related pathology. Overall, 40% of the mice from the WA 1X and WA 2X groups survived to the end of the 6-month observation period and from the 32 GT recipients of the secondary transplant, 25 mice were observable for more than two months. A slower mortality rate was observed in the WA 1X and WA 2X treated groups, when compared to WKO untreated animals.

At sacrifice, chimerism and VCN detected in BM and spleen, were overall comparable with values observed in primary transplants, demonstrating engraftment of transduced HSPC in

secondary recipients. Wiskott-Aldrich syndrome transgene expression was confirmed in the GT-treated mice by reverse transcriptase polymerase chain reaction (PCR). Thymic lymphomas were present in three GT-treated animals (1x WA 1X (8.3%) and 2x WA 2X (15.4%) groups) but molecular analysis confirmed all were of host origin and did not contain LVV sequences.

In conclusion, serial transplantation experiments followed up for a cumulative period of 10 months showed no evidence of GT-related toxicity or tumorigenicity and confirmed the long-term hematopoietic repopulating capacity of w1.6W_mut6 LVV transduced stem cells.

In the clinical development programme of Telethon003, no cases of malignant clonal expansion or AEs indicative of oncogenic transformation have been reported and there has been no evidence of aberrant clonal behaviour based on insertion site analysis (Section 4,3, Module 2.7.4). In Study 201228, molecular analysis indicated polyclonal hematopoietic reconstitution, and stable engraftment of the vector marked clones. No sustained clonal expansions were observed in any of the subjects. No signs of insertional mutagenesis were detected. Overall data indicate a stable engraftment of vector marked clones long-term, supporting the safety and efficacy of treatment [Module 5.3.5.1, 201222 Clinical Study Report (CSR); Module 5.3.5.4, 206258/205029 CSR; Module 5.3.5.2, 205756 CSR]. In Study OTL-103-4, none of the insertion sites identified in the PB or BM cells of the subjects showed a contribution of > 30% of the total retrieved insertion sites (Section 11.1.3.4 in Study OTL-103-4 CSR).

These findings are similar to those reported in other Fondazione Telethon LVV based HSPC GT trials for metachromatic leukodystrophy (MLD), Beta-Thalassemia, Mucopolysaccharidosis Type I, and X-linked chronic granulomatous disease (Biffi, 2013; Aiuti, 2013). In 2020, Libmeldy, an LVV-based HSPC GT was approved by EMA and in 2024 by FDA (Lenmeldy) for the treatment of early onset MLD. The WAS LVV is similar to the vector used for the manufacture of Libmeldy. Both vectors use the same lentiviral backbone, and the only difference between the WAS and the MLD LVV is the expression cassette containing the promoter and the transgene.

In previous GT studies for WAS using LTR intact gRV vectors, several patients developed leukaemia as a result of insertional mutagenesis (Braun, 2014). Although no such event was reported after lentiviral GT for WAS, Telethon003 subjects are monitored for abnormal clonal proliferation. The lack of transcriptionally active LTR of the SIN lentiviral backbone, combined with a moderately active internal promoter (WAS endogenous promoter) to drive transgene expression is a major safety advantage of the vector. The risk of insertional mutagenesis and consequent tumorigenicity following administration of Telethon003 was also evaluated in a number of in vitro and in vivo pre-clinical studies, with no evidence of tumorigenicity observed. In the clinical studies and the EAP (long-term follow-up to 13 years after GT), no clonal expansion or leukaemia development has been observed. Integration site analysis following Telethon003 administration shows that the vector insertions are polyclonal and does not reveal any clonal expansion or enrichment for proto-oncogenes. Long-term follow-up of participants in the clinical studies and EAP continues, in order to monitor this potential risk.

Importantly, the promoters and regulatory elements of Fondazione Telethon vectors are derived from human (not viral) sequences and are specifically designed to have limited enhancer activity on neighbouring genes thereby mitigating the potential for safety concerns.

On 16 February 2021, Bluebird Bio announced that the company placed its Phase 1/2 (HGB-206) and Phase 3 (HGB-210) studies of LentiGlobin GT for sickle cell disease (SCD) (bb1111) on a temporary suspension due to a reported Suspected Unexpected Serious Adverse Reaction (SUSAR) of acute myeloid leukaemia (AML). However, following in depth assessment of the available data, the company reported that it was very unlikely the event to be related to the

BB305 LVV citing instead significant chromosomal abnormalities and mutations in genes typically associated with the development of AML.

On August 9, 2021, Bluebird Bio disclosed a patient administered with its elivaldogene autotemcel (eli-cel) GT for cerebral adrenoleukodystrophy (CALD) was subsequently diagnosed with MDS that may be attributable to treatment. In October 2024 Bluebird Bio reported blood cancers in other 6 patients treated with their LVV transduced CD34+ product for CALD: overall six of the seven cases of hematological malignancies were diagnosed as myelodysplastic syndrome (MDS), arising between 14 and 92 months after Skysona treatment. The other patient was diagnosed with acute myeloid leukemia (AML) at 57 months. Six patients were subsequently treated with stem cell transplantation, which led to one death due to graft-versus-host disease. The single AML patient remains alive and has shown good response to the transplant.

The Lenti-D vector used to produce the eli-cel DP employed the MND gRV enhancer/promotor region to drive therapeutic transgene expression. This class of viral promoters are known to carry the risk of transactivating, or "turning on," neighbouring genes. Fondazione Telethon does not use the MND promoter, or any other viral regulatory elements, to produce any of their DPs. Importantly, no events of clonal dominance associated with integrations into the MECOM locus have been observed. The type of regulatory elements used across the Fondazione Telethon portfolio are human, non-viral promoters that lack enhancer activity. The vectors are tested prior to use in the clinic to minimize the potential for dysregulating nearby genes. No AEs related to vector integration have currently been reported in any of the patients treated with Fondazione Telethon's lentiviral hematopoietic stem cell (HSC) gene therapies.

Risk-benefit impact

Malignancy due to insertional oncogenesis would be serious and could potentially be life-threatening.

The benefit of Waskyra as a treatment for severe WAS outweighs the theoretical risk of malignancy due to insertional oncogenesis that is mitigated by the use of a SIN LVV with minimal enhancer activity.

The potential serious safety concern of malignancy due to insertional oncogenesis will be further monitored in ongoing and planned studies including Study OTL-103-4 and a study to monitor the long-term safety of Telethon003 up to 15 years after treatment (Part III.2) as well as via routine pharmacovigilance activities.

Important Potential Risk 2

Engraftment failure

Engraftment failure is defined as failure to reach an absolute neutrophil count (ANC) >500 neutrophils/µL associated with no evidence of BM recovery (i.e., hypocellular marrow) by day +60. After conditioning, all subjects in study 201228, the EAP and OTL-103-4 experienced severe neutropenia (neutrophil count <0.5 x 10^9/L [<500/µL]). To date, none of the subjects in the clinical development programme have had haematological engraftment failure.

In study OTL-103-4, vector-transduced HSPCs were considered to have engrafted if gene corrected cells were detected in the PB of subjects 6 months post Telethon003. Adequate engraftment was defined as detection of ≥ 10% gene-marked PB T lymphocytes or PB mononuclear cells (when T cells alone were not sufficient); this was equivalent to 0.1 VCN/cell. Adequate engraftment at 2 years was defined as percentage of LVV-transduced BM-derived colony forming units (CFUs) (%LVV in BM-CFU) ≥ 10% and VCN/cell of ≥ 0.04 in BM-derived $CD34^+$ cells. This value was based on clinical experience in other GT trials, which

demonstrated 4% average long-term engraftment of autologous HSPC transduced cells with retroviral vectors in the BM of paediatric patients with adenosine deaminase-deficient severe combined immunodeficiency and receiving reduced intensity, non-myeloablative conditioning (Aiuti, 2009b; Cicalese, 2016).

In addition, multilineage engraftment was evaluated in BM and/or PB subpopulations (BM subpopulations: glycophorin A^+ [$GlyA^+$], $CD15^+$, $CD3^+$, $CD19^+$, $CD56^+$, $CD34^+$; PB subpopulations: $CD15^+$, $CD19^+$, $CD3^+$, $CD56^+$). If data on PB $CD3^+$ were not available, the average of PB $CD4^+$ and PB $CD8^+$ T cells were calculated and reported as T cell engraftment. Adequate multilineage engraftment was defined as ≥ 4% gene-marked cells in all the available cells (equivalent to 0.04 VCN/cell).

The predefined sustained engraftment parameters were met in all patients in the Telethon003 development programme and EAPs.

None of the subjects in Study 201228 and Study OTL-103-4, or any patients in the EAP, met the pre-specified definition of engraftment failure requiring infusion of the HSPC backup. (Study 201228 CSR; Study OTL-103-4 CSR, Expanded Access Program).

Risk-benefit impact

Engraftment failure is an important potential risk as it would have a negative impact on the patient in terms of WAS disease progression and associated morbidities and mortality. However, the benefit of Waskyra outweighs the potential risk of engraftment failure that has not been observed in the clinical trials and that can be managed in clinical practice through patient monitoring and infusion of non-transduced back-up cells, which are collected during the harvest/mobilisation and kept in storage untransduced.

The important potential risk of engraftment failure will be further characterised after the treatment of new patients via routine pharmacovigilance activities and the provision of educational/safety advice tools in the context of clinical trials open to recruitment (Study OTL-103-04) and a via planned PASS to monitor the long-term safety of Telethon003 (Part III.2).

Missing information 1

Long-term safety data

Waskyra is an ex vivo autologous $CD34^+$ haematopoietic stem cell GT administered once only as a single dose for the treatment of patients with WAS. Following successful and stable engraftment, the effects of the product are expected to be life-long.

The median duration of follow-up in the Integrated Safety Set (N=27) available at the time of this RMP was 5.666 years (range 0.37 to 13.26 years). The median duration of follow-up was longer in study 201228 than for study OTL-103-4. More specifically, the median duration of follow-up for study 201228 was 11.118 years minimum and range 8.01-13.26 years while for study OTL-103-4 the median duration of follow up was 5.020 years minimum and range 2.31-5.43 years

As long-term safety data are limited this is recognised as an area of missing information.

Risk-benefit impact

The long-term safety of Waskyra will be further characterised via routine pharmacovigilance activities and the provision of educational/safety advice tools as well as in multiple ongoing and

planned studies including, Study OTL-103-4 and a planned Long Term Follow Up study to monitor the long-term safety of Telethon003 (Part III.2).

SVII.2 New safety concerns and reclassification with a submission of an updated RMP

Not applicable

SVII.3 Details of important identified risks, important potential risks, and missing information

SVII.3.1. Presentation of important identified risks and important potential risks

Important Identified Risk

None

Important Potential Risk 1

Malignancy due to insertional oncogenesis

Potential mechanisms:

Insertional oncogenesis is a potential safety concern with integrating viruses in gene-modified cell therapies (Marcucci, 2018).

Insertional oncogenesis has been seen in previous GT studies but has been associated with the use of LTR intact gRV vectors where the mechanism of oncogenesis was directly related to the enhancer activity of the viral LTR which was able to transactivate neighbouring growth promoting genes. Gammaretroviral vectors demonstrate integrations accumulating at transcription start sites of gene-coding regions, the majority of which have previously been described as proto-oncogenes including *MECOM* and *LMO2* (Braun, 2014).

The vector used to deliver the WAS gene to target HSPCs is an HIV-1-derived replication deficient SIN third generation LVV containing a 1.6kb fragment of the human WAS endogenous promoter and the human WAS cDNA thus reducing the risk of activating neighbouring genes following insertion.

Lentiviral vectors preferentially integrate into active gene loci, but with patterns that are less mutagenic than those observed with other retroviral vectors (Hutson, 2013; Schroder, 2002; Schambach, 2013).

Evidence source(s) and strength of evidence:

Vector insertion site analysis (VISA) was performed to interrogate the integration profile of the WAS LVV (w1.6W_mut6) in healthy donor or WAS patient $CD34^+$ cells in vitro, and in cells from healthy donors in vivo after engraftment in Rag2−/−Il2rγc−/− mice (Scaramuzza, 2013). The insertion profiles in vitro and in vivo confirmed the preference of LVV for transcriptional units inside genes and without any bias for promoter regions. The pattern of insertions was highly polyclonal both in vitro in human $CD34^+$ cells from HD and WAS patients and in vivo upon infusion in Rag2−/−Il2rγc−/− mice. No differences were observed from the use of different vector batches or with 1- or 2-hit transduction protocols.

Further evaluation of the potential genotoxicity of WAS LVV integration in vivo was conducted using a high-throughput VITA technique to tag and identify vector DNA in the SV129 WKO mouse genome following ex vivo transduction and transplant of murine WAS-/- Lin- cells into lethally irradiated recipients (Mantovani, 2009). This study also confirmed a polyclonal pattern of integration in transcription units within genes and no hotspots of insertion.

In vivo carcinogenicity risk was further evaluated by transplanting Lin- cells purified from the BM of tumor prone Cdkn2a-/- mice and transduced ex vivo with LVV expressing GFP in place of WAS, into WT mice (FVB/N.129-cdkn2atm2Rdp strain) (Montini, 2006). No evidence of tumor acceleration was observed. In contrast, mice given cells transduced with a γRV showed a dose-dependent acceleration of tumor onset and reduced survival. Integration site analysis of pre- and post-transplant cells transduced with GFP LVV showed no bias for any gene class or in vivo cell selection. It was concluded that the integration of prototypical SIN LVV has low genotoxic potential.

In previous GT studies for WAS using LTR intact gRV vectors, several patients developed leukaemia as a result of insertional mutagenesis (Braun, 2014). Although no such event was reported after lentiviral GT for WAS, Telethon003 subjects are monitored for abnormal clonal proliferation. The lack of transcriptionally active LTR of the SIN lentiviral backbone, combined with a moderately active internal promoter to drive transgene expression, such as the WASP endogenous promoter, is a major safety advantage of the vector. The risk of insertional mutagenesis and consequent tumorigenicity following administration of Telethon003 was also evaluated in a number of in vitro and in vivo pre-clinical studies, with no evidence of tumorigenicity observed. In the clinical studies and the EAP (long-term follow-up to 10.5 years after GT), no clonal expansion or leukaemia development has been observed.

Importantly, the promoters and regulatory elements of Fondazione Telethon vectors are derived from human (not viral) sequences and are specifically designed to have limited enhancer activity on neighbouring genes thereby mitigating the potential for safety concerns.

On 16 February 2021, Bluebird Bio announced that the company placed its Phase 1/2 (HGB-206) and Phase 3 (HGB-210) studies of LentiGlobin GT for SCD (bb1111) on a temporary suspension due to a reported SUSAR of acute myeloid leukaemia (AML). However, following in depth assessment of the available data, the company reported that it was very unlikely the event to be related to the BB305 LVV citing instead significant chromosomal abnormalities and mutations in genes typically associated with the development of AML.

On August 9, 2021, Bluebird Bio disclosed a patient administered with its elivaldogene autotemcel (eli-cel) GT for CALD was subsequently diagnosed with MDS that may be attributable to treatment. In October 2024 Bluebird Bio reported blood cancers in other 6 patients treated with their LVV transduced CD34+ product for CALD: overall six of the seven cases of hematological malignancies were diagnosed as myelodysplastic syndrome (MDS), arising between 14 and 92 months after Skysona treatment. The other patient was diagnosed with acute myeloid leukemia (AML) at 57 months. Six patients were subsequently treated with stem cell transplantation, which led to one death due to graft-versus-host disease. The single AML patient remains alive and has shown good response to the transplant.

The Lenti-D vector used to produce the eli-cel DP employed the MND gRV enhancer/promotor region to drive therapeutic transgene expression. This class of viral promoters are known to carry the risk of transactivating, or “turning on,” neighbouring genes. Fondazione Telethon does not use the MND promoter, or any other viral regulatory elements, to produce any of their DPs. Importantly, no events of clonal dominance associated with integrations into the MECOM locus have been observed. The type of regulatory elements used across the Fondazione Telethon

portfolio are human, non-viral promoters that lack enhancer activity. The vectors are tested prior to use in the clinic to minimize the potential for dysregulating nearby genes. No AEs related to vector integration have currently been reported in any of the patients treated with Fondazione Telethon's lentiviral HSC gene therapies

In 2020, Libmeldy, an LVV-based HSPC GT was approved by EMA for the treatment of early onset MLD. The WAS LVV is similar to the vector used for the manufacture of Libmeldy. Both vectors use the same lentiviral backbone, and the only difference between the WAS and the MLD LVV is the expression cassette containing the promoter and the transgene. Integration site analysis in patient cells following Telethon003 administration shows that the vector insertions are polyclonal and do not reveal any clonal expansion, nor enrichment for proto-oncogenes. Long-term follow-up of participants in the clinical studies and EAP continues, in order to monitor this potential risk.

Characterisation of the risk:

Waskyra consists of $CD34^+$ cells transduced ex vivo with a LVV which integrates permanently into the host genome. The risk of insertional mutagenesis and consequent tumorigenicity has been evaluated in several in vitro and in vivo non-clinical studies.

The vector selected for use in the Telethon003 clinical development program (WAS LVV) is an HIV-1-derived replication deficient SIN third generation LVV containing a 1.6kb fragment of the human WAS endogenous promoter and the human WAS cDNA. In the context of an LVV, this promoter appears weaker in driving WASP mRNA expression than others, such as elongation factor 1 alpha (EF1alpha). However, it appears to lead to optimal expression of WASP (Charrier, 2007), thereby suggesting it achieves optimal regulation of the WAS gene expression. The endogenous WAS promoter also restricts transgene expression to hematopoietic cells (Dupré, 2004), thus limiting the possibility of transgene toxicity to this system.

Published preliminary data from ongoing GT clinical studies using the WAS LVV indicate that reconstitution with gene-corrected cells is highly polyclonal, with intermittent progenitor activity and no evident abnormal clonal expansion. No association between recurrent integration near specific cancer-associated genes and cell amplification or persistence has been detected (Aiuti, 2013; Ferrua, 2019).

Risk factors and risk groups:

Factors thought to be important in contributing to the risk of oncogenesis (EMA/CAT/190186/2012):

a) Vector design (including backbone and regulatory elements)
b) Insertion profile
c) Vector copy number (VCN)
d) Transgene product
e) Target cell population/organ
f) Risk of malignancy for the underlying disease

Preventability:

The risk of insertional oncogenesis is minimised by the SIN design of the LVV used for Waskyra which abolishes LTR promoter activity and transgene expression. The risk can be further minimised through increasing healthcare professional and patient and parent/carer awareness and patient monitoring.

Healthcare professionals are informed that Waskyra contains $CD34^+$ cells which have been genetically modified with an LVV. The Waskyra SmPC advises that no cases of leukaemia or lymphoma have been reported during the clinical development of Telethon003. Nevertheless, there is a theoretical risk of leukaemia or lymphoma after Waskyra treatment and in the event that leukaemia or lymphoma is detected in any patient who received Waskyra, Fondazione Telethon should be contacted to obtain instructions on collection of blood samples for ISA.

Similarly, patients and their parents/carers are advised in the Waskyra PL that inserting a new gene into the DNA could theoretically cause blood cancers (leukaemia and lymphoma), although no patients have developed leukaemia or lymphoma in clinical trials with Telethon003. After treatment with Waskyra the patient will be asked to enrol in a LTFU study for up to 15 years after treatment to better understand the long-term safety and efficacy of Waskyra and during the long-term follow-up, the patient will be monitored for any signs of leukaemia or lymphoma.

If leukaemia/lymphoma is detected, blood samples for ISA should be collected.

Impact on the risk-benefit balance of the product:

Malignancy due to insertional oncogenesis would be serious and could potentially be life-threatening. To date, no cases of malignancy or AEs indicative of oncogenic transformation have been reported with Telethon003, or with other gene therapies using LVV in the Fondazione Telethon's development program. Abnormal haematopoietic clonal expansion was monitored by clinical and laboratory surveillance, T cell receptor repertoire study, and BM examination. No evidence of abnormal clonal proliferation was observed. The molecular analysis of the LVV integration sites in BM and PB samples collected after treatment indicated polyclonal haematopoietic reconstitution and the absence of clonal expansion or enrichment for proto-oncogenes in all subjects. No signs of insertional mutagenesis were detected.

The benefit of Waskyra as a treatment for the severe WAS outweighs the potential risk of malignancy due to insertional oncogenesis that can be managed in clinical practice through patient monitoring and standard of care treatment, including possible rescue allogeneic-HSCT if needed.

Public health impact:

Taking into account the rarity of WAS (Module SI), the potential public health impact is considered to be low.

Important Potential Risk 2

Engraftment failure

Potential mechanisms:

The procedure to manufacture Waskyra involves the harvest of $CD34^+$ HSPC enrichment from patient mPB and transduction with a WAS LVV, which inserts 1 or more copies of the human WAS cDNA into the cell's genome so that modified cells become capable of expressing a functional protein.

Successful therapy with Waskyra requires RIC with busulfan and fludarabine.

When administered to the patient following the protocol above, the genetically modified cells expressing a functioning WAS protein engraft and repopulate the haematopoietic compartment. Sustained engraftment of the transduced cells is therefore essential for the success of treatment.

Engraftment failure was defined as ANC ≤ 500 cells/μL and no evidence of BM recovery (ie, hypocellular BM) at Day 60. No subject showed evidence of engraftment failure.

In the clinical development program, Telethon003 was infused intravenously in subjects with WAS after administration of anti-CD20 monoclonal antibody (rituximab) and a RIC using busulfan and fludarabine. The RIC was selected based on the assumption that a stable mixed chimerism with gene corrected and uncorrected cells, both in BM and PB, would be sufficient to provide clinical benefit, with a reduced regimen-related toxicity. Busulfan was administered every 6 hours, from Day -3 to Day -1. The dose of busulfan was adjusted to avoid excessive toxicity or insufficient exposure. Initially, the target AUC range was 4500-6000 ng/mL*h per dose, equivalent to a cumulative target AUC range of 36000-48000 ng/mL*h. This value is equivalent to 40-53% of the myeloablative dose exposure (90000 ng/mL*h).

After treatment of 6 subjects in study 201228, the clinical protocol was amended to set the total target AUC to 48000 +-10% ng/mL*h (range: 43200-52800). This decision was made as the available data suggested that a cumulative estimated AUC <40000 ng/mL*h may be associated with a lower myeloid engraftment in BM and a longer period of platelet transfusion dependence. Rituximab was administered as pre-treatment at a dose of 375 mg/m2 in a single dose, on Day -22. Fludarabine was administered at a total dose of 60 mg/m2, equal to 30 mg/m2/day, on the first two days of busulfan administration, and. During the course of the EAP, a protocol amendment was implemented allowing for a wash-out period of 24-48 hours between the last dose of busulfan and the administration of the GT product.

Evidence source(s) and strength of evidence:

Stable engraftment in immunodeficient Rag2-/-Il2rγc-/- neonatal mice was confirmed with positive WASP expression for as long as 16 months following primary transplant or for a cumulative period of 10 months including 6 months of secondary transplant.

Engraftment is characterized by the presence of genetically modified cells in the BM and PB compartments. The main indicator of gene correction is detection of the *WAS* LVV sequences in the HSPCs and their progeny. The VCN, which is the mean number of integrated copies of the vector sequences per cell genome, was measured using PCR-based methods in DNA samples extracted from BM and PB cell populations at various timepoints post-treatment.

All participants in the Efficacy population achieved adequate engraftment of BM $CD34^+$ and/or PB $CD3^+$ cells by 6 months postTelethon003. Median time to engraftment was 32.0 days (95% CI: 30.0-33.0). Median time to engraftment of PB $CD3^+$ cells alone was 91.0 days (95% CI: 61.0-180.0; Table 2.7.3.3.1.5).

For two participants in Study OTL-103-4, the reported time to engraftment of PB $CD3^+$ was 376 and 426 days, respectively. However, the actual time to engraftment in these participants could

not be assessed precisely due to the long time intervals between the 6-month and the 12-month assessments or due to missed follow-up visits during the COVID-19 pandemic (Section 11.1.2.3 in Study OTL-103-4 CSR).

To date, there is no evidence of engraftment failure in any patient treated with Telethon003.

Characterisation of the risk:

Engraftment failure is defined as failure to reach an ANC >500 neutrophils/µL associated with no evidence of BM recovery (i.e., hypocellular marrow) by day +60. After conditioning, all subjects in study 201228, the EAP and OTL-103-4 experienced severe neutropenia (neutrophil count <0.5 x 10^9/L [<500/µL]). No subject showed evidence of engraftment failure.
One subject in study OTL-103-4 met the pre-specified definition of prolonged aplasia (ANC <0.5 x 10^9/L [<500/µL] at Day 60) and received G-CSF due to an event of autoimmune neutropenia leading to low neutrophil count while his BM was not hypocellular. Granulocyte-colony stimulating factor was also administered to one subject in 201228 within that time period. Neither event was considered to be an SAE by the investigator or by Fondazione Telethon.

Adequate engraftment of genetically corrected cells, defined as ≥0.04 VCN/cell (equivalent to 4% of cells being genetically corrected assuming a VCN of 1) in each subpopulation, was achieved in all BM and PB cell subpopulations for all participants with evaluable data at Year 1 (n=25, 95% CI: 86.7-100% [For $CD34^+$ BM only: n=25, 95% CI: 86.7-100%) and all participants with evaluable data at Year 2 (n=21; 95% CI: 84.5-100%) (Table 2.7.3.3.1.1).

Risk factors and risk groups:

Graft failure is a serious complication of allogeneic HSCT, defined as either lack of initial engraftment of donor cells (primary graft failure) or loss of donor cells after initial engraftment (secondary graft failure or graft rejection). Graft failure can be life-threatening and may require a second rescue transplant procedure if a suitable donor is available. The literature review reported a graft failure rate of 11.0% (Pallas, 2022) while the 5.1% of patients in the Burroughs et al. study required a second HSCT procedure (Burroughs, 2020).

No engraftment failure was observed after treatment with Telethon003, and all Telethon003 subjects (100%) showed sustained engraftment of HSPCs in the blood and BM after having received one GT treatment.

The Telethon003 clinical development programme involved the administration of a RIC regimen, in contrast to the more toxic MAC that is generally administered before HSCT. Haematological recovery after RIC regimen was generally achieved by 2 months after GT. Twelve of the 14 subjects achieved haematological reconstitution (ANC > 500 cells/µL) by 60 days post Telethon003 infusion. Two subjects had ANC < 500 cells/µL at this timepoint; however, these 2 subjects had no evidence of hypocellular bone marrow and achieved adequate engraftment of gene-corrected cells, defined as ≥ 0.04 VCN/cell in BM $CD34^+$ cells, by this timepoint and were thus not engraftment failures.

Preventability:

Waskyra specifications for % $CD34^+$ and cell viability are set based upon supporting quality and non-clinical data to ensure that viable $CD34^+$ cells will meet the intended dose. In the clinical

trials engraftment failure is defined as failure to reach an ANC >500 neutrophils/µL associated with no evidence of BM recovery (i.e., no hypocellular marrow) by day +60.

The Waskyra SmPC advises that Waskyra must be administered in a Fondazione Telethon QTC with experience in HSCT. Healthcare professionals are advised that in clinical trials, no patients failed to engraft BM, as measured by neutrophil engraftment (N=27). Patients should be monitored for signs and symptoms of cytopenia for at least 6 weeks after infusion. Red blood cells should be monitored according to medical judgment until engraftment of these cells and recovery are achieved. Supportive transfusion of red cells and/or platelets should be given according to medical judgement and institutional practice. If cytopenia persists beyond six to seven weeks, despite the use of granulocyte mobilising agents, the non-transduced back-up cells should be infused. If cytopenia persists despite infusion of non-transduced back-up stem cells, alternative treatments should be considered.

The Waskyra PL informs the patient / carer that if the modified stem cells do not take hold (engraft) in the patient's body, the doctor may give them an infusion of their original stem cells that were collected and stored as a backup.

Impact on the risk-benefit balance of the product:

Engraftment failure would have a negative impact on the patient in terms of WAS disease progression and associated mortality. However, the benefit of Waskyra as a treatment for the life-threatening disease, outweighs the potential risk of engraftment failure that has not been observed in the Waskyra clinical development programme and that can be managed in clinical practice through patient monitoring and infusion of the back-up followed by allogeneic-HSCT, if needed, in case of Waskyra failure.

Public health impact:

There is no public health impact of engraftment failure.

SVII.3.2. Presentation of the missing information

Missing information 1:

Long term safety data

Evidence source:

Waskyra is an ex vivo autologous $CD34^+$ haematopoietic stem cell GT administered once only as a single dose for the treatment of patients with WAS. Following successful and stable engraftment in the patient, the effects of the product are expected to be life-long.

A total of 26 subjects were undergoing follow-up at the time of data cut-off for the integrated analyses.

Overall, adequate multilineage engraftment of genetically corrected cells (defined as ≥0.04 VCN/cell; equivalent to 4% assuming a VCN of 1) was observed and sustained over time in all tested BM lineages, including stem cells ($CD34^+$), erythroid cells ($GlyA^+$), granulocytes ($CD15^+$), T cells ($CD3^+$), B cells ($CD19^+$) and natural killer cells ($CD56^+$) scoring of the LVV^+ BM-

derived CFUs confirmed the presence of corrected progenitor cells in the BM up to 8 years after cell infusion.

Population in need of further characterisation:

The long-term safety of Waskyra will be further characterised in ongoing study OTL-103-4. All patients treated with Telethon003/Waskyra from clinical development program, Early Access Scheme and commercial settings will be invited to enrol in a LTFU study to monitor the long-term safety of Waskyra (Part III.2, Annex 5).

Part II: Module SVIII - Summary of the safety concerns

Table SVIII.1: 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

PART III: PHARMACOVIGILANCE PLAN (INCLUDING POST-AUTHORISATION SAFETY STUDIES)

III.1 Routine pharmacovigilance activities

Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:

- **Specific adverse reaction follow-up questionnaires for <safety concerns>:**

  None.

- **Other forms of routine pharmacovigilance activities for <safety concerns>:**

  None.

III.2 Additional pharmacovigilance activities

Waskyra Long Term Follow up study
WAS-TLT003-01

Advanced therapy medicinal products, including gene therapy, require long-term patient follow-up for products based on LVVs, due to their oncogenic potential and the persistence of gene modified cells after treatment. Long-term follow-up observations are extended assessments that continue some of the scheduled observations of a clinical trial past the follow-up period. LTFU studies, lasting up to 15 years from treatment with the gene therapy product, may identify long-term risks to the patients receiving treatment.

This LTFU study is designed to collect long-term safety and efficacy data from WAS patients previously treated with Telethon003, as part of the Telethon003 clinical development program and expanded access program (EAP) such as Hospital Exemption and Compassionate Use and from patients treated under 648/1996 Italian law or any other early access program. Moreover, this LTFU is aimed at collecting long-term safety and efficacy data also from all WAS patients treated.

No investigational drug product will be administered in the study.

The study is planned to recruit 40 patients who will be followed on up to 15 years after the Telethon003 administration.

It is expected that the 26 patients who have already been treated in the clinical development phase and in the EAP and are in the follow up phase will all enter in the LTFU study to continue be monitored for safety and efficacy until they achieved the 15th years after Telethon003 administration. The remaining 14 patients to be included in the LTFU study are patients treated under 648/1996 Italian law (five have already been treated) or patients treated in a commercial setting.

The sample size of 40 is not statistically driven; it is mainly based on the following consideration:

-Good safety profile of Telethon003: no engraftment failure, no malignancy due to insertional oncogenesis, no patient positive for replicant competent lentivirus

- Already available long-term follow up data: The median duration of the 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) with eight patients who have already been monitored for more than 8 years
- Patients treated under 648/1996 Italian law and patients treated in a commercial setting may be considered a single population, being all be treated with the cryo formulation and managed in a clinical setting outside a clinical development program.
- Burder to recruit and manage the follow up for an elevated number of patients. It is expected to recruit and treat 3 patients/year hence the LTFU study will last about 18 years after the potential marketing authorization
- Should the number of the recruited commercial patients not be considered enough, the LTFU can be amended to increase the sample size

Study: OTL-103-4:

Purpose of the study: The primary objective of the study is to evaluate the safety and efficacy of the cryopreserved formulation of Telethon003 (previously OTL-103) in 10 subjects at 12 months for bleeding events and from 6 to 18 months for severe infections prior to being transferred into a long-term follow-up study to be monitored for 15 years after GT.

The study is being conducted in two clinical centres, at the Pediatric Clinical Research Unit/Pediatric Immuno-hematology and Bone Marrow Transplantation Unit, Ospedale San Raffaele, Milan, Italy and at the Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, GA 30322, United States

Ten participants were enrolled in the study and 10 received Telethon003. As of the cut-off date of the last interim analysis (17 jan 2025), all 10 participants had 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). The WAS gene mutation was classified as severe in all but one participant, for whom severity of the mutation was not known. All participants had a history of bleeding events, recurrent infection, and skin disorders.

The objective of this study was to evaluate the efficacy and safety of the cryopreserved formulation of Telethon003. This formulation has the advantage of allowing full quality control testing of the product before infusion and will also facilitate the distribution of Telethon003 to a wider patient population. In this interim analysis , the cryopreserved formulation of Telethon003 has shown clinical and biological efficacy in participants with WAS, as indicated by adequate and stable engraftment of gene-modified PB and BM cells, increased WASP expression in PB cells, restored immune function and increased platelet count, resulting in significant reduction of severe infections and moderate/severe bleeding events and positive responses to vaccinations. These efficacy outcomes were maintained in all participants over time, whilst sustained treatment with platelet transfusions and IgRT could be ceased in all evaluable participants, and sustained antimicrobial treatment could be ceased in all but one participant who received such treatment during the study. This led to significant reduction of hospitalizations and allowed participants to stop living in a protective environment, attend school or kindergarten, and start sports activities, whilst maintaining a life essentially free of severe infections and bleedings. The cryopreserved

formulation of Telethon003 was well tolerated in all participants, with no Telethon003-related AEs being reported. All participants are still alive. There have been no reports of development of insertional mutagenesis or abnormal clonal proliferation, immune response to transgene, graft failure, or graft-versus-host disease. Follow-up of this study is ongoing which will yield more evidence to confirm the efficacy and safety of the cryopreserved formulation of Telethon003. No safety issues related to the drug product were reported.

The study is still ongoing and it is going to be completed in late 2027 and be reported by June 2028.

III.3 Summary Table of additional Pharmacovigilance activities

Table Part III.1: Planned additional pharmacovigilance activities

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)	• Interim analysis	Protocol submission: Within 3 months of the Commission Decision Interim analyses: Every two years. Final CSR: 31 December 2046

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
		• 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	• Final study report	

Table Part III.2: Planned additional pharmacovigilance activities

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). **Ongoing**	Primary: • to evaluate the clinical efficacy of the cryopreserved formulation of Telethon003 at 12 months for bleeding events 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	• Malignancies due to insertional oncogenesis • Engraftment failure • Long-term safety data	• Interim analysis	Dec 2021 July 2024 Apr 2025
			• Final study report	Jun 2028

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
	• 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			

Part IV: Plans for post-authorisation efficacy studies

Study Status	Summary of objectives	Efficacy uncertainties addressed	Milestones	Due dates
Efficacy studies which are conditions of the marketing authorisation				
None				
Efficacy studies which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				

PART V: RISK MINIMISATION MEASURES (INCLUDING EVALUATION OF THE EFFECTIVENESS OF RISK MINIMISATION ACTIVITIES)

Risk Minimisation Plan

V.1. Routine Risk Minimisation Measures

Table Part V.1: Description of routine risk minimisation measures by safety concern

Safety concern	**Routine risk minimisation activities** *(in addition to routine signal detection and periodic aggregate safety report assessment)*
Malignancy due to insertional oncogenesis (Important potential risk 1)	Routine risk minimisation communication: • 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 there have been no cases of leukaemia or lymphoma in clinical studies in SmPC section 4.4 • Information that no patients have developed leukaemia or lymphoma in clinical trials with Waskyra in PL section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: • Warning that Waskyra may theoretically cause leukaemia or lymphoma with instructions on tissue/blood sample collection if malignancy occurs in SmPC section 4.4 and request to provide advance consent for use of samples for integration site analysis (ISA) in case of malignancy is diagnosed in the future • 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 • Commitment to performing ISA in all reported cases of malignancy suspected to be related to Waskyra, regardless of the patient's participation in a clinical trial • Interaction with treating physicians and pathology departments to facilitate access to relevant samples upon notification of malignancy Other routine risk minimisation measures beyond the Product Information:

Safety concern	**Routine risk minimisation activities** *(in addition to routine signal detection and periodic aggregate safety report assessment)*
	• Legal status: Medicinal product subject to restricted medical prescription
Engraftment failure (Important potential risk 2)	Routine risk communication: • Information that no patients failed to engraft bone marrow in SmPC sections 4.4 and 5.1 • 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 Routine risk minimisation activities recommending specific clinical measures to address the risk: • Instructions to obtain a CD34+ stem cell back-up for use as rescue treatment in SmPC section 4.2 • Warning that in case of cytopenia symptoms, red blood cells and platelet counts should be monitored until engraftment of these cells and recovery are achieved in SmPC section 4.4 • Guidance that in case of engraftment failure, the non-transduced back-up cells should be infused in SmPC section 4.4 • Guidance that if the modified stem cells do not take hold (engraft) in the patient's body, the doctor may give an infusion of the backup original stem cells in PL section 2 Other routine risk minimisation measures beyond the Product Information: • Legal status: Medicinal product subject to restricted medical prescription
Long-term safety data (Missing information 1)	Routine risk communication: • Information on the duration of patient follow-up in the clinical studies in SmPC section 5.1 Routine risk minimisation activities recommending specific clinical measures to address the risk: • 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 Other routine risk minimisation measures beyond the Product Information:

Safety concern	Routine risk minimisation activities *(in addition to routine signal detection and periodic aggregate safety report assessment)*
	• Legal status: Medicinal product subject to restricted medical prescription

V.2. Additional Risk Minimisation Measures

Additional risk minimisation 1 – Healthcare professionals educational/safety advice tools

Educational/safety advice tools will be provided to healthcare professionals, the patients and/or their parents/carers.

Fondazione Telethon will provide educational/safety advice tools (Guide for risk minimisation for HCP; Guide for handling and method of administration) to healthcare professionals involved in Waskyra treatment of a patient with WAS (Annex 6).
Objectives: To facilitate informed decision-making by healthcare professionals and patients, parents/carers based on the known benefits and risks of Waskyra and to minimise the risks to patients.
To ensure that Waskyra, an autologous $CD34^+$ HSPC GT, is administered only to the donor patient from whom the original stem cells originated with detailed guidance on handling and the method of administration for Waskyra.

- To highlight the important risks of Waskyra to healthcare professionals with guidance on how to minimise the risks, including the potential risk of leukaemia/lymphoma and the need for monitoring treated patients for signs and symptoms of oncogenic transformation, leukaemia or lymphoma and the potential risks of engraftment failure.

To make physicians aware of the importance of monitoring and long-term follow-up, and to inform them about the LTFU study.

Rationale for the additional risk minimisation activity:

These materials supplement information in the SmPC by describing the safety concerns and actions to take to minimise the important risks associated with Waskyra and to highlight the importance of monitoring and long-term follow-up which will further characterise the important risks and long-term safety.

Target audience and planned distribution path:

Healthcare professionals working at QTCs involved in the treatment of WAS with Waskyra. HCPs will receive training on the guide for risk minimisation for HCP as part of the centre qualification process.

Plans to evaluate the effectiveness of the interventions and criteria for success:

Section XVI.B.4 of Guideline on GVP XVI revision 2 (EMA, 2017), states that: “In rare circumstances when it is justified that the assessment of outcomes indicators is unfeasible (e.g.,

inadequate number of exposed patients, very rare AEs), the effectiveness evaluation may be based exclusively on the careful interpretation of data on process indicators".

The expected number of patients to be exposed to Waskyra in post-marketing settings/clinical studies and the number of qualified centres involved in the administration of the product is expected to be very low and overall considered inadequate for the implementation of a study with sufficient statistical power to capture the effectiveness of the material itself.
Moreover, as reported above, section 4.2 of the SmPC reports that Waskyra "must be administered in a QTC with experience in Haematopoietic Stem Cell Transplantation (HSCT)".
Therefore, the educational/safety advice tools will target professionals who are already highly specialised in the management, treatment and care of patients undergoing HSCT.
Given the above, Fondazione Telethon considers that the above measures, are sufficient to ensure adequate penetration of the material itself among treating HCPs**.** A similar method for evaluating the effectiveness of additional risk minimisation measures was endorsed for a recently authorised product with a similar technological therapeutic approach to Waskyra (Libmeldy, atidarsagene autotemcel).

Criteria for success:

- Objective A: All treating physicians at QTCs should receive educational/safety advice tools.
- Objective B: Sample-handling compliance in case of malignancy.

The above process indicators will be presented in the PSURs.

Additional risk minimisation 2 – Patient and parent/carer information pack

Fondazione Telethon will provide educational/safety advice tools and communication materials in the local language (Guide for risk minimisation for patient/caregiver, Patient card, patient information leaflet) to patients and their parents/carers (Annex 6).

Objectives: To facilitate informed decision-making by patients or by their parents/carers based on the known benefits and risks of Waskyra and to minimize the risks to patients including what actions to take and when and how to contact their specialist doctor in case of side effects and how to report adverse drug reactions.
To highlight the need for the patient - or their parent/carer - to carry the Patient Card to inform any treating healthcare professional that they were treated with Waskyra. The **Patient Card can be provided in two or more copies upon request;** one for the patient, and one for the caregiver, given the young age of the indicated patient population. Additional copies will be provided upon request.
To explain the need for regular monitoring and to report any symptoms or concerns to the specialist doctor treating the child.
To make patients aware of the importance of regular monitoring and inform them about the objectives and duration of the LTFU study and that enrolment in the LTFU study will not be a prerequisite for treatment with Waskyra.
Moreover, the Patient Card reports information that the patient has received Waskyra, a GT medicinal product for WAS, and should not donate blood, organs, tissues, and cells for transplantation. The card also clearly states that there is a possibility of false positive results on

certain commercial HIV tests. The Patient Card contains the treating physician details, highlights that Waskyra is subject to additional monitoring and how to report adverse reactions.

Rationale for the additional risk minimisation activity: These materials supplement information in the PL by describing the safety concerns and actions to take to minimise the important risks. Information about the long-term study is provided to encourage patient participation.
Target audience and planned distribution path: Patients with WAS eligible for Waskyra and their parents/carers.

Plans to evaluate the effectiveness of the interventions and criteria for success: The distribution of the educational/safety advice tools for patients will be captured as part of the enrolment process by Fondazione Telethon QTC.

Criteria for success:
All patients treated with Waskyra should receive educational/safety advice tools.

The above process indicators will be presented in the PSURs

Additional risk minimisation 3 – Risk minimisation control programme

Waskyra will only be available at QTCs with experience in HSCT to ensure that:
• this therapy is only delivered by healthcare professionals who have been adequately trained on the proper use of the product, and
• the traceability of the patients' cells and manufactured medicinal product is maintained between the QTCs and the manufacturing site.

Objectives: To ensure that Waskyra will only be available through Fondazione Telethon QTC so that only eligible patients are treated with Telethon003 and to ensure maintained traceability of Waskyra throughout the manufacturing process to administration.

Rationale for the additional risk minimisation activity: The manufacturing process of Waskyra is complex with multiple steps involved (Figure 2 Flow Chart of the Logistics of Therapy). As Waskyra is an autologous product it is essential to ensure the product is traceable from the autologous cell procurement stage back to the same patient for treatment with medicinal product.

Target audience and planned distribution path: Treating physicians, manufacturers and QTC staff.

Plans to evaluate the effectiveness of the interventions and criteria for success: The compliance with risk minimisation control programme is an unconditional premise for Waskyra prescription, manufacturing, and DP administration to eligible WAS patients. The risk minimisation control programme will be regularly monitored by Fondazione Telethon.

Removal of additional risk minimisation activities

Not applicable.

V.3 Summary of risk minimisation measures

Table Part V.3: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

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	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
	• Educational/safety advice tools for patients • Provision of extra Patient Cards Other routine risk minimisation measures beyond the Product Information: • Legal status: Medicinal product subject to restricted medical prescription	
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	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
	• 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: • 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	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
	Other routine risk minimisation measures beyond the Product Information: • Legal status: Medicinal product subject to restricted medical prescription	

PART VI: SUMMARY OF THE RISK MANAGEMENT PLAN

Summary of risk management plan for Waskyra (Etuvetidigene autotemcel**)**

The RMP details important risks of Waskyra, how these risks can be minimised, and how more information will be obtained about Waskyra's risks and uncertainties (missing information).

Waskyra's summary of product characteristics (SmPC) and its package leaflet give essential information to healthcare professionals and patients on how Waskyra should be used.

This summary of the RMP for Waskyra should be read in the context of all this information including the assessment report of the evaluation and its plain-language summary, all which is part of the European Public Assessment Report (EPAR).

Important new concerns or changes to the current ones will be included in updates of Waskyra's RMP.

I. The medicine and what it is used for

Waskyra is indicated for the treatment of patients aged 6 months and older with severe Wiskott-Aldrich Syndrome (WAS) who have a mutation in the *WAS* gene and for whom no suitable human leukocyte antigen (HLA)-matched related stem cell donor is available. Each patient specific infusion bag contains a $CD34^+$ cell enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced ex vivo using a lentiviral vector (LVV) encoding the human WAS complementary deoxyribonucleic acid (cDNA) and is given as an intravenous (IV) infusion.

II. Risks associated with the medicine and activities to minimise or further characterise the risks

Important risks of Waskyra, together with measures to minimise such risks and the proposed studies for learning more about Waskyra's risks, are outlined below.

Measures to minimise the risks identified for medicinal products can be:

- Specific information, such as warnings, precautions, and advice on correct use, in the package leaflet and SmPC addressed to patients and healthcare professionals;
- Important advice on the medicine's packaging;
- The authorised pack size — the amount of medicine in a pack is chosen so to ensure that the medicine is used correctly;
- The medicine's legal status — the way a medicine is supplied to the patient (e.g., with or without prescription) can help to minimise its risks.

Together, these measures constitute *routine risk minimisation* measures.

In the case of Waskyra, these measures are supplemented with *additional risk minimisation measures* mentioned under relevant important risks, below.

In addition to these measures, information about adverse reactions is collected continuously and regularly analysed, including PSUR assessment - so that immediate action can be taken, as necessary. These measures constitute *routine pharmacovigilance activities*.

If important information that may affect the safe use of Waskyra is not yet available, it is listed under 'missing information' below.

II.A List of important risks and missing information

Important risks of Waskyra are risks that need special risk management activities to further investigate or minimise the risk, so that the medicinal product can be safely administered. Important risks can be regarded as identified or potential. Identified risks would be concerns for which there would be sufficient proof of a link with the use of Waskyra. Potential risks are concerns for which an association with the use of this medicine is possible based on available data, but this association has not been established yet and needs further evaluation. Missing information refers to information on the safety of the medicinal product that is currently missing and needs to be collected (e.g., on the long-term use of the medicine).

List of important risks and missing information	
Important identified risks	None
Important potential risks	Malignancy due to insertional oncogenesis Engraftment failure
Missing information	Long-term safety data

II.B Summary of important risks

Important identified risks

None.

Important potential risk: Malignancy due to insertional oncogenesis	
Evidence for linking the risk to the medicine	Vector insertion site analysis (VISA) was performed to interrogate the integration profile of the WAS LVV (w1.6W_mut6) in healthy donor or WAS patient $CD34^+$ cells in vitro, and in cells from healthy donors in vivo after engraftment in Rag2−/−Il2rγc−/− mice (Scaramuzza, 2013). The insertion profiles in vitro and in vivo confirmed the preference of LVV for transcriptional units inside genes and without any bias for promoter regions. The pattern of insertions was highly polyclonal both in vitro in human $CD34^+$ cells from HD and WAS patients and in vivo upon infusion in Rag2−/−Il2rγc−/− mice. No differences were observed

	from the use of different vector batches or with 1- or 2-hit transduction protocols. Further evaluation of the potential genotoxicity of WAS LVV integration in vivo was conducted using a high-throughput VITA technique to tag and identify vector DNA in the SV129 WKO mouse genome following ex vivo transduction and transplant of murine WAS-/- Lin- cells into lethally irradiated recipients (Mantovani, 2009). This study also confirmed a polyclonal pattern of integration in transcription units within genes and no hotspots of insertion. In vivo carcinogenicity risk was further evaluated by transplanting Lin- cells purified from the BM of tumor prone Cdkn2a-/- mice and transduced ex vivo with LVV expressing GFP in place of WAS, into WT mice (FVB/N.129-cdkn2atm2Rdp strain) (Montini, 2006). No evidence of tumor acceleration was observed. In contrast, mice given cells transduced with a γRV showed a dose-dependent acceleration of tumor onset and reduced survival. Integration site analysis of pre- and post-transplant cells transduced with GFP LVV showed no bias for any gene class or in vivo cell selection. It was concluded that the integration of prototypical SIN LVV has low genotoxic potential. Integration site analysis in patient cells following Telethon003 administration shows that the vector insertions are polyclonal and do not reveal any clonal expansion, nor enrichment for proto-oncogenes. In previous GT studies for WAS using LTR intact gRV vectors, several patients developed leukaemia as a result of insertional mutagenesis (Braun, 2014). Although no such event was reported after lentiviral GT for WAS, Telethon003 subjects are monitored for abnormal clonal proliferation. The lack of transcriptionally active LTR of the SIN lentiviral backbone, combined with a moderately active internal promoter to drive transgene expression, such as the WASP endogenous promoter, is a major safety advantage of the vector. The risk of insertional mutagenesis and consequent tumorigenicity following administration of Telethon003 was also evaluated in a number of in vitro and in vivo pre-clinical studies, with no evidence of tumorigenicity observed. In the clinical studies and the EAP (long-term follow-up to

	10.5 years after GT), no clonal expansion or leukaemia development has been observed. Importantly, the promoters and regulatory elements of Fondazione Telethon vectors are derived from human (not viral) sequences and are specifically designed to have limited enhancer activity on neighbouring genes thereby mitigating the potential for safety concerns.
Risk factors and risk groups	Factors thought to be important in contributing to the risk of oncogenesis (EMA/CAT/190186/2012): a) Vector design (including backbone and regulatory elements) b) Insertion profile c) Vector copy number (VCN) d) Transgene product e) Target cell population/organ f) Risk of malignancy for the underlying disease
Risk minimisation measures	Routine risk minimisation measures • 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 there have been no cases of leukaemia or lymphoma in clinical studies in SmPC section 4.4 • Information that no patients have developed leukaemia or lymphoma in clinical trials with Waskyra in PL section 2 • Warning that Waskyra may theoretically cause leukaemia or lymphoma with instructions on tissue/ blood sample collection if malignancy occurs in SmPC section 4.4 • Warning that the patient will be asked to enrol in follow up study for up to 15 years and will be monitored for any signs of blood cancer because of the theoretical cancer risk in PL section 2 Additional risk minimisation measures • Educational/safety advice tools for healthcare professionals • Educational/safety advice tools for patients

Additional pharmacovigilance activities	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) See section II.C of this summary for an overview of the post-authorisation development plan.

Important potential risk: Engraftment failure	
Evidence for linking the risk to the medicine	Engraftment failure has been defined as ANC ≤ 500 cells/µL and no evidence of BM recovery (i.e., hypocellular BM) at Day 60. No subject showed evidence of engraftment failure. Stable engraftment in immunodeficient Rag2-/-Il2rγc-/- neonatal mice was confirmed with positive WASP expression for as long as 16 months following primary transplant or for a cumulative period of 10 months including 6 months of secondary transplant. All subjects in the integrated Efficacy population achieved adequate engraftment of BM $CD34^+$ and/or PB $CD3^+$ cells by Day 33 post Telethon003. Median time to engraftment was 32.5 days (95% CI: 30 - 33). Median time to engraftment of PB $CD3^+$ cells alone was 94 days (95% CI: 34 – 187). For 2 subjects in Study OTL-103-4, estimated time to engraftment of PB $CD3^+$ was 376 days and 426 days, respectively. This is likely longer than the actual time to engraftment, which could not be assessed in these subjects because of the long-time intervals between assessments or missed follow-up visits during the COVID-19 pandemic. At Year 1, VCN were comparable in both BM $CD34^+$ and PB $CD3^+$ T cells from subjects who received Telethon003 sourced from mPB stem cells and those who received Telethon003 sourced from BM stem cells. At Year 2, VCN in both BM $CD34^+$ and PB $CD3^+$ T cells was higher in the subjects who received the fresh formulation of Telethon003 sourced from mPB

	stem cells; however, data were only available from 2 subjects who received Telethon003 sourced from mPB stem cells, as no subjects in Study OTL-103-4 have yet reached the 2-year timepoint. Overall, the VCN in the BM $CD34^+$ cells and the PB $CD3^+$ cells were not markedly different whether the DP was made from BM or mPB starting material.
Risk factors and risk groups	Graft failure is a serious complication of allogeneic HSCT, defined as either lack of initial engraftment of donor cells (primary graft failure) or loss of donor cells after initial engraftment (secondary graft failure or graft rejection). Graft failure can be life-threatening and may require a second rescue transplant procedure if a suitable donor is available. The literature review reported a graft failure rate of 11.0% (Pallas, 2022) while the 5.1% of patients in the Burroughs et al. study required a second HSCT procedure (Burroughs, 2020). No engraftment failure was observed after Telethon003 infusion, and all Telethon003 subjects (100%) showed sustained engraftment of HSPCs in the blood and BM after having received one GT treatment. The Telethon003 clinical development programme involved the administration of a RIC regimen, in contrast to the more toxic MAC that is generally administered before HSCT. Haematological recovery after RIC regimen was generally resolved by 2 months after GT. Twelve of the 14 subjects achieved haematological reconstitution (ANC > 500 cells/µL) by 60 days post OTL- 103. Two subjects had ANC < 500 cells/µL at this timepoint; however, these 2 subjects had no evidence of hypocellular bone marrow and achieved adequate engraftment of gene-corrected cells, defined as ≥ 0.04 VCN/cell in BM $CD34^+$ cells, by this timepoint and were thus not engraftment failures.
Risk minimisation measures	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 engraftment 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
Additional pharmacovigilance activities	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) See section II.C of this summary for an overview of the post-authorisation development plan.

Missing information: Long term safety information	
Evidence for linking the risk to the medicine	Waskyra is an ex vivo autologous $CD34^+$ haematopoietic stem cell GT administered once only as a single dose for the treatment of patients with WAS. Following successful and stable engraftment in the patient, the effects of the product are expected to be life-long. A total of 26 subjects were undergoing follow-up at the time of data cut-off for the integrated analyses.

	Overall, adequate multilineage engraftment of genetically corrected cells (defined as ≥0.04 VCN/cell; equivalent to 4% assuming a VCN of 1) was observed and sustained over time in all tested BM lineages, including stem cells ($CD34^+$), erythroid cells ($GlyA^+$), granulocytes ($CD15^+$), T cells ($CD3^+$), B cells ($CD19^+$) and natural killer cells ($CD56^+$) scoring of the LVV^+ BM- derived CFUs confirmed the presence of corrected progenitor cells in the BM up to 8 years after cell infusion.
Risk minimisation measures	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 4.4 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
Additional pharmacovigilance activities	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) See section II.C of this summary for an overview of the post-authorisation development plan.

II.C Post-authorisation development plan

II.C.1 Studies which are conditions of the marketing authorisation

The following studies are conditions of the marketing authorisation:

Study: 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)

Purpose of the study: This LTFU study is designed to collect long-term safety and efficacy data from WAS patients previously treated with Telethon003, as part of the Telethon003 clinical development program and expanded access program (EAP) such as Hospital Exemption and Compassionate Use and from patients treated under 648/1996 Italian law or any other early access program. Moreover, this LTFU is aimed at collecting long-term safety and efficacy data also from WAS patients treated under early access or EAP after the approval of this study or possible future commercial setting. No investigational drug product will be administered in the study.

II.C.2 Other studies in post-authorisation development plan

Study short name

Study: OTL-103-4:

Purpose of the study: The primary objective of the study is to evaluate the safety and efficacy of the cryopreserved formulation of Telethon003 (previously OTL-103) in 10 subjects at 12 months for bleeding events and from 6 to 18 months for severe infections prior to being transferred into a long-term follow-up study to be monitored for 15 years after GT.

PART VII: ANNEXES

Table of contents

Annex 1 – EudraVigilance Interface

EEA-QPPV Contact Details

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The EEA-QPPV will be contactable on the above details, in case of unavailability the following person will act as the EEA-QPPV's back-up.

Deputy EEA-QPPV

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Annex 2 – Tabulated summary of planned, ongoing, and completed pharmacovigilance study programme

Table 1: Planned and ongoing studies

Study	Summary of objectives	Safety concerns addressed	Protocol link Milestones
Study OTL-103-4 A Clinical Study to Evaluate the Use of a Cryopreserved Formulation of Telethon003 (previously OTL-103) in Subjects with WAS. Category 3 **Ongoing**	To evaluate the safety and efficacy of the cryopreserved formulation -To evaluate the engraftment and biological efficacy of the cryopreserved formulation of Telethon003.	• Malignancies due to insertional oncogenesis • Engraftment failure • Long-term safety data	Study OTL-103-4 FPFV: 21-Jan-2019 Interim analysis: Dec 2021 Jult 2024 Apr 2025 Final study report: June 2028
Long-term Follow-up Study 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) Category 1 **Approved, Initiated**	Long term follow-up	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)	Protocol submission: Within 3 months of the Commission Decision Interim analyses: Every two years. Final CSR: 31 December 2046

Study	Summary of objectives	Safety concerns addressed	Protocol link Milestones
		• 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	

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Annex 2 Table 2: Completed studies

Study	Summary of objectives	Safety concerns addressed	Date of Final Study Report submission Link to report
Study 201228 A phase I/II clinical trial of hematopoietic stem cell gene therapy for the Wiskott-Aldrich Syndrome (TIGET-WAS) Category 3 **Closed**	To assess the safety and efficacy of Telethon003 (previously OTL-103)	• Malignancies due to insertional oncogenesis • Engraftment failure • Long-term safety data	Study 201228 First Patient First Visit (FPFV): 20-Apr-2010 Interim reports: 10-Jan-2017 17-Feb-2022 Final study report: September 2024
CUP 206257 Compassionate Use Program for haemato- poietic stem cell gene therapy for Wiskott- Aldrich Syndrome Category 3 **Closed**	Compassionate treatment	• Malignancies due to insertional oncogenesis • Engraftment failure • Long-term safety data	CUP 206257 FPFV: 29-Nov-2016 Final study report: September 2024
WAS-HE-GT Hematopoietic stem cell gene therapy for the Wiskott-Aldrich Syndrome (WAS) prepared on non-routine basis and pursuant to a prescription individually	Compassionate treatment	• Malignancies due to insertional oncogenesis • Engraftment failure • Long-term safety data	WAS-HE-GT (all protocols identical other than patient number) FPFV: 14-Dec-2015 Final study report: September 2024

Study	Summary of objectives	Safety concerns addressed	Date of Final Study Report submission Link to report
targeted for the patient coded -WAS-HE01 -WAS-HE02 -WAS-HE03 (Individual patients assigned to unique protocols; however, all protocols contain the same language) **Closed**			

Annex 3 – Protocols for proposed, ongoing and completed studies in the pharmacovigilance plan

Table of contents

Part A: Requested protocols of studies in the Pharmacovigilance Plan, submitted for regulatory review with this updated version of the RMP

Not applicable

Part B: Requested amendments of previously approved protocols of studies in the Pharmacovigilance Plan, submitted for regulatory review with this updated version of the RMP

Not applicable

Part C: Previously agreed protocols for ongoing studies and final protocols not reviewed by the competent authority

Approved protocols:

Study 201228

Study OTL-103-4

Compassionate Use Program 206257

Hospital Exemption 205030

Early Access Scheme in Italy under national Law 648/96

Study: 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)

Final protocols not reviewed or not approved

Not applicable.

Annex 4 – Specific adverse drug reaction follow-up forms

Not applicable.

Annex 5 – Protocols for proposed and ongoing studies in RMP part IV

Not applicable.

Annex 6 – Details of proposed additional risk minimisation activities

Prior to launch of Waskyra in each Member State, Fondazione Telethon will agree the content and format of the educational/safety advice tools and risk minimisation control programme with the National Competent Authority. The educational and risk minimisation control programme will be aimed at providing information on the safe use of Waskyra.

The MAH shall ensure that in each Member State where Waskyra is marketed, all healthcare professionals and patients/carers who are expected to prescribe, dispense and/or use Waskyra have access to/are provided with the following educational package

- HCP educational/safety advice tools
- Patient information pack (local language)

The HCP educational/safety advice tools should contain:

- The Summary of Product Characteristics
- Guide for risk minimisation for HCP
- Guide for handling and method of administration

The Guide for risk minimisation for HCP shall contain the following key elements:

- Warning that there is a theoretical possibility that the treatment with Waskyra may be associated with the risk of insertional mutagenesis, potentially leading to development of malignancy. All patients should receive monitoring for signs and symptoms of oncogenic transformation, leukaemia, or lymphoma or solid organ malignancy; and must be advised on the symptoms and signs of leukaemia or lymphoma or solid organ malignancy; and to seek immediate medical attention if they develop any of the symptoms.
- Warning about the potential risk of engraftment failure and the need to monitor patients.
- Information on Long term Follow Up study and what it will involve.
- Malignancy Work up Checklist
- Recommendation of the important considerations to discuss with patients and/or carers about Waskyra:
    - Potential risks of a treatment with Waskyra.
    - Signs of any malignancy such as leukaemia/lymphoma or solid organ malignancy and what action to take.
    - Content of the patient and parent/carer guide.
    - The need to carry the Patient Card and to show it to every healthcare professional.
    - The importance of regular monitoring and long-term follow-up.

- Provision of contact details for reporting all suspected adverse reactions and to include the individual medicinal product lot number which can be found within the Patient Card.

The Guide for handling and method of administration for Waskyra for healthcare professionals shall contain the following key elements:

- Guidance that Waskyra must be administered in a QTC with experience in HSCT.
- Instructions on the precautions to be taken before handling or administering Waskyra.
- Instructions for receiving and storing Waskyra.
- Instructions to check Waskyra prior to administration.
- Instructions for the thawing of Waskyra.
- Provision of contact details for reporting all suspected adverse reactions and to include the individual medicinal product lot number which can be found within the Patient Card.

The patient information pack shall contain:

- The Package leaflet
- Guide for risk minimisation for patient/caregiver
- The Patient card: the **Patient Card can be provided in two or more copies upon request**; one for the patient, and one for the caregiver, given the young age of the indicated patient population.

Guide for risk minimisation for patient/caregiver shall contain the following key messages:

- Warning to monitor the patient for symptoms of leukaemia or lymphoma and to contact the specialist doctor immediately in case of any symptoms as there is a small risk that a patient may develop leukaemia or lymphoma or solid organ malignancy. The specialist doctor will check the patient's blood for any signs of leukaemia or lymphoma or solid organ malignancy during the routine yearly check-ups, which will continue after treatment.
- Guidance about the need for the patient or their parent/carer to carry the Patient Card to inform any treating healthcare professional that the child was treated with Waskyra.
- Guidance on the importance of regular monitoring and to report any symptoms or concerns to the specialist doctor treating the child.
- Information about the Long Term Follow Up study and the purpose of the study.

- Provision of contact details for reporting any side effects or symptoms of the patient and what a medicine subject to additional monitoring (▼) means.
- **Patient card with the following key messages:**
- Statement that the patient was treated with Waskyra, with the medicinal product lot number and treatment date to ensure traceability as per the Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products (EMEA/149995/2008).
- Contact details of the treating physician.
- Information on the possibility of false positivity of certain commercial HIV tests because of Waskyra.
- Statement that the patient was treated with GT and should not donate blood, organs, tissues, or cells.
- Details on reporting of adverse reactions and that Waskyra is subject to additional monitoring▼.
- Contact details where a healthcare professional can receive further information.

Risk minimisation control programme

Fondazione Telethon shall ensure that in each Member State where Waskyra is marketed, a system aimed to control distribution to Waskyra is ensured by routine risk minimisation measures. The following requirements need to be fulfilled before the product is prescribed, manufactured, dispensed, and used:

Waskyra shall only be available through QTCs to ensure traceability of the patient's cells and manufactured DP between the treating hospital and manufacturing site. The selection of the QTCs shall be conducted in collaboration with national health authorities as appropriate. The HCPs will receive training on the physician educational/safety advice tools as part of the centre qualification process.

Annex 7 – Other supporting data (including referenced material)

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Annex 8 – Summary of changes to the risk management plan over time

Version	Approval date Procedure	Change
0.0	Not applicable	Not applicable
0.1	Not applicable	SVII.1.2 • Important Potential Risk 2 Engraftment failure added. • Missing Information 2 Clinical data on female patients added. SVIII.3.1 • Important Potential Risk 2 Engraftment failure added. • Missing Information 2 Clinical data on female patients added. Table SVIII updated. Table Part III.1 updated. Table Part III.2 updated. Table Part V.1 updated. V.2 Additional Risk Minimisation Measures updated. V.3. Summary of Risk Minimisation Measures updated. Part II.A List of Important Risks and Missing Information updated. Part II.B Summary of Important Risks updated. Annex 2 updated. Annex 3 updated. Annex 6 updated.
0.2	Not applicable	Table SIV.2: Exposure of special populations included or not in clinical trial development programmes Added “Female population” and “Adults”.
		SVII.1.1. Risks not considered important for inclusion in the list of safety concerns in the RMP: added “Adults population.”
		Table Part III.1: Planned additional pharmacovigilance activities Added in the column “Safety concerns addressed”: • to evaluate the risk of engraftment failure • to evaluate the efficacy/ safety profile on female and adult patients;
		Table Part III.2: Planned additional pharmacovigilance activities Added in the column “Safety concerns addressed”: •Malignancies due to insertional oncogenesis

Version	Approval date Procedure	Change
		•Engraftment failure •Long-term safety data
		Table Part V.1: Description of routine risk minimisation measures by safety concern Deleted in the column "Safety concerns addressed": ~~Additional risk minimisation activities~~ ~~•Educational/safety advice tools for healthcare professionals~~ ~~Educational/safety advice tools for patients~~ ~~Additional risk minimisation measures:~~ ~~•Educational/safety advice tools for healthcare professionals.~~ ~~•Guide for risk minimisation for patient/caregiver.~~ ~~•Controlled distribution~~
		V.2. Additional Risk Minimisation Measures Changed some wording according to "D180 List of Outstanding Issues".
		Table Part V.3: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern Added: •Clinical experience in female patients is extremely limited; treatment decisions should be guided by specialist centres and all any treated female subjects should be invited to participate to the post-authorisation study registry up to 15 years after gene therapy.
		II.B Summary of important risks Missing information: Long term safety information: changed some wording according to "D180 List of Outstanding Issues".
		Annex 2 – Tabulated summary of planned, ongoing, and completed pharmacovigilance study programme Table 1: Planned and ongoing studies - Long term follow up: added in the column "Safety concerns addressed": 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)

Version	Approval date Procedure	Change
		•to evaluate the overall survival up to 15 years of follow-up post treatment with gene therapy •to evaluate the risk of engraftment failure •to evaluate the efficacy/ safety profile on female and adult patients Deleted: ~~To characterize the long-term safety of the gene therapy treatment.~~
		Annex 6 – Details of proposed additional risk minimisation activities: changed some wording according to "D180 List of Outstanding Issues".
0.3	Not applicable	Updated indication in: Indication in EEA
		The safety concern 'Clinical data on female patients' has been deleted from 'missing information' in the RMP (Table Part III.1, Part V, Annex 2 and Annex 6.
		Two typos have been corrected in Annex 6
0.4	Not applicable	Aligned with SmPC
0.5	Not applicable	Alignment of PASS LTFU study description and milestones across SmPC and RMP