COVER PAGE

Official Study Title: Efficacy and safety of a cryopreserved formulation of autologous CD34+ hematopoietic stem cells transduced *ex vivo* with EFS lentiviral vector encoding for human ADA gene in subjects with Severe Combined Immunodeficiency due to Adenosine Deaminase deficiency.

Protocol Version 5.0, 28 March 2018

NCT02999984

PROTOCOL AMENDMENT 4: OTL-101-4 (28 March 2018)

EFFICACY AND SAFETY OF A CRYOPRESERVED FORMULATION OF AUTOLOGOUS CD34+ HEMATOPOIETIC STEM CELLS TRANSDUCED EX VIVO WITH EFS LENTIVIRAL VECTOR ENCODING FOR HUMAN ADA GENE IN SUBJECTS WITH SEVERE COMBINED IMMUNODEFICIENCY DUE TO ADENOSINE DEAMINASE DEFICIENCY

STUDY PROTOCOL
STUDY number: OTL-101-4
PRODUCT: A CRYOPRESERVED FORMULATION OF AUTOLOGOUS CD34+ HEMATOPOIETIC STEM CELLS TRANSDUCED EX VIVO WITH EFS LENTIVIRAL VECTOR ENCODING FOR THE HUMAN ADA GENE (OTL-101)
[IND number: 15440]

Original Protocol, Version 1.0: 24 October 2016
Protocol Amendment 1, Version 2.0: 18 January 2017
Protocol Amendment 2, Version 3.0: 05 May 2017
Protocol Amendment 3, Version 4.0: 08 September 2017
Protocol Amendment 4, Version 5.0: 28 March 2018

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PROTOCOL SIGNATURES

Investigator Agreement and Signature:

I have read and agree to the protocol titled "Efficacy and safety of a cryopreserved formulation of autologous CD34+ hematopoietic stem cells transduced *ex vivo* with EFS lentiviral vector encoding for human ADA gene in subjects with severe combined immunodeficiency due to adenosine deaminase deficiency" (protocol number: OTL-101-4). I am aware of my responsibilities as an Investigator under the guidelines of Good Clinical Practice (GCP)[1], local regulations (as applicable) and the study protocol. I agree to conduct the study according to these guidelines and to appropriately direct and assist the staff under my control, who will be involved in the study.

[redacted]

1 ICH Harmonised Tripartite Guideline E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) Step 5, adopted by CPMP July 1996/Food and Drug Administration Code of Federal Regulations for Good Clinical Practices Parts 50, 56, 312 , 314/ European Directives 2001/20/EC and 2005/28/EC as implemented into national legislation

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SYNOPSIS

Sponsor	Orchard Therapeutics, Ltd.
Investigational Product	A cryopreserved formulation of autologous CD34+ hematopoietic stem cells transduced *ex vivo* with Elongation Factor 1α Short form (EFS) lentiviral vector (LV) encoding for the human adenosine deaminase deficiency (ADA) gene (OTL-101)
Study Title:	Efficacy and safety of a cryopreserved formulation of autologous CD34+ hematopoietic stem cells transduced *ex vivo* with EFS lentiviral vector encoding for human *ADA gene* in subjects with Severe Combined Immunodeficiency (SCID) due to Adenosine Deaminase Deficiency
Number of Planned Centres	1
Planned Study Period	December 2016 to December 2020
Study Objectives:	**Primary Study Objectives:** There are two primary objectives for this study aimed to determine; 1. The success of treatment at the subject level ("responder analysis") 6 months post OTL-101 infusion, defined as: a) Evidence of erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units), b) Evidence of immune reconstitution (absolute number of CD3 cells ≥200/mm^3), c) Detectable gene-marked granulocytes by differential polymerase chain reaction (dPCR)/qPCR (≥1/10,000 cells). This 6 month data will be used to compare the proportion of responders among subjects treated with the cryopreserved population with a comparable population obtained from one of the ongoing Phase I/II studies using the fresh formulation. 2. The overall survival and event free survival at 12 months among ADA-SCID subjects treated with a cryopreserved formulation of OTL-101. Overall survival is defined as the proportion of subjects alive. Event-free survival is defined as the proportion of subjects alive with no "event", an "event" being the resumption of PEG-ADA ERT or the need for a rescue allogenic HSCT, or death. **Secondary Study Objectives** The secondary objectives to be evaluated among ADA-SCID subjects treated with a cryopreserved formulation of OTL-101 include confirmation of the overall survival and event free survival at 24 months, safety evaluation including infection rates, quality of life and immune response. **Exploratory Study Objectives** Exploratory objectives include the measurement of biological correlates of efficacy including; percentage of gene marking in peripheral blood granulocytes, vector integration analysis, quantification of clonal diversity of vector integrants, T-cell receptor

	excision circle (TREC) and fluorescence-activated cell sorting (FACS) for T-cell receptor (TCR) V-β family use, ADA enzyme activity in erythrocytes, total adenine nucleotides in erythrocytes, and immune reconstitution..
Study Design:	This is a prospective, non-randomized, single-cohort, longitudinal, single-center, clinical study designed to assess the efficacy and safety of OTL-101 cryopreserved formulation administered in ADA-SCID subjects aged between ≥30 days to <18 years of age, who are not eligible for an HLA-matched sibling/family donor and meeting the inclusion/exclusion criteria. This study aims to recruit 10 evaluable subjects. The aim of this clinical study is also to assess the success of treatment at the subject level ("responder analysis") 6 months post OTL-101 infusion, using predictive criteria for overall survival and event free survival and to compare data obtained from clinical studies using the fresh formulation of OTL-101. Eligible subjects will be hospitalized to undergo the harvesting of autologous CD34+ cells. A backup harvest of non-transduced CD34+ cells will be obtained during the harvesting procedure to be used in the event of i) product damage during the thawing of the OTL-101 product that would prevent infusion of OTL-101 although the patient has already been conditioned or ii) lack of engraftment/hematopoietic reconstitution 42 days post infusion of OTL-101. A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of OTL-101 infusion. OTL-101 is a cryopreserved formulation of autologous CD34+ hematopoietic stem cells transduced ex vivo with EFS LV encoding for the human ADA gene (OTL 101). To enable the release of OTL-101 for infusion, the cryopreserved OTL-101 product must meet various quality control criteria for safety, identity, viability, purity and potency. If OTL-101 meets the acceptance criteria and is released, the subjects will be readmitted for conditioning with Busulfan. The OTL-101 product will be infused after a minimal interval of at least 24 hours following the completion of Busulfan administration. Subjects may remain as an in-patient following autologous transplantation for approximately three to 35 days. For subjects who have successfully received the OTL-101 product, PEG-ADA ERT use will be evaluated at the 1 Month follow-up visit (30 days post treatment ± 7 days) and discontinued at Day 30 (-3/+15) after the transplant. Hematologic reconstitution will be assessed at Day 42 (± 7 days), and in the event of no reconstitution the backup HSC sample will be administered, if the investigator believes this is in the subject's best interest. The subjects will then be seen at regular intervals to review their history, perform examinations and draw blood samples at Months 3, 6 and 9 (all ± 2 weeks) as well as Months

	12, 18 and 24 (all ± 4 weeks). Any medically-indicated interventions, including the need to reinstate PEG-ADA ERT, will be assessed at all follow-up visits after Month 1. Hematopoietic reconstitution will be assessed again at the Month 6 visit and if there is still no evidence of cellular recovery, the subject will be given ERT and/or HSCT, if available and deemed appropriate by the Investigator. After the Month 24 visit, the subjects will have completed the study and may enter a long term registry. The study will be overseen by an independent Data Safety Monitoring Board (DSMB), who will review the nature and severity of emerging safety data during the study. The DSMB will also liaise with the Principal Investigator directly to ensure the safety of participants during the study. The Investigator, subjects and their families will be informed about any significant emerging safety data.
Study Population:	This study aims to enrol 10 eligible subjects aged ≥30 days and <18 years, with a diagnosis of ADA-SCID and ineligible for matched family allogeneic bone marrow transplantation
Duration of Participation:	The total study duration for each subject is estimated to be up to 2 years and 4 months.
Study Evaluations:	Primary endpoints for this study include: 1. Evaluation of OTL-101 at 6 months post infusion, in support of CMC assessing comparability of cryopreserved and fresh formulations, evaluation of therapy success for each subject based on the following parameters and their thresholds: • Erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units), • Absolute CD3+ T-cell counts ≥200/mm^3, and • Peripheral blood samples positive for vector sequences by qPCR(≥1/10,000 cells). Subjects must meet all three criteria. Subjects not meeting these criteria will be designated a failure (non-responder) and will be withdrawn from the study. This data will be used to compare subject data from one of the ongoing Phase I/II studies using the fresh formulation. 2. Evaluate the overall survival and event free survival 12 months post OTL-101 infusion. Overall survival is defined as the proportion of subjects alive. Event-free survival is defined as the proportion of subjects alive with no "event", an "event" being the resumption of PEG-ADA ERT or the need for a rescue allogenic HSCT, or death. Secondary endpoints include: • Evaluate the overall survival and event free survival 24 months post OTL-101 infusion. • Immunoglobulin Replacement therapies prior to and after gene therapy.

	• Performance outcomes and quality of life will be measured by the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance and ability to practice sports, respectively. • Rates of severe infections/ opportunistic infectious episodes, defined as infections requiring hospitalization or prolonging hospitalization and/or documented infections by opportunistic pathogens (i.e. interstitial pneumonia, intractable diarrhoea). • Response to tetanus vaccination. • Immune reconstitution: T and B cell reconstitution. The exploratory endpoints will include laboratory correlates of efficacy that will be used to assess the level of gene correction, engraftment and immune reconstitution as exploratory endpoints..
Safety Evaluations:	The safety and tolerability of OTL-101 will be assessed throughout the study by evaluating AEs, clinical laboratory test results, vital signs measurements, electrocardiogram (ECG) and physical examination results, and concomitant medication usage.
Statistical Considerations:	The statistical analyses of efficacy data will be performed in three stages: 1. The first analysis will compare the success/failure data 6-months post OTL-101 infusion between fresh and cryopreserved OTL-101 formulations. The success/failure of the OTL-101 will be defined by the three critera listed in the primary objectives. This data will be compared with available data from one of the ongoing Phase I/II studies using the OTL-101 fresh formulation. This will support the CMC comparability data between OTL-101 cryopreserved and fresh formulations. Secondary and exploratory endpoints will also be described. 2. The second analysis will determine the 12 month overall survival and event free survival for all subjects. Secondary and exploratory endpoints, as well as baseline characteristics, subject disposition, and safety data will also be described. 3. The third and final analysis will be performed to determine the overall survival and event free survival for all subjects 24 months post OTL-101 infusion. Secondary and exploratory endpoints, as well as baseline characteristics, subject disposition, safety and Busulfan pharmacokinetic (PK) data will also be described. Efficacy analyses will be primarily descriptive in nature.

Table 1: Schedule of Events – Screening to Day 24

Study Period	**Screening**	**Pre-harvest Assessment**	**Bone Marrow Harvest**	**Baseline**	**Conditioning**		**OTL-101 Treatment**	**Short-term Follow-up**						
Visit number	1	2[1]	2	3[1]	3	3	3	3	3	3	3	3	3	3
Assessment number		A	B	C	D	E	F	G	H	I	J	K	L	M
Visit window								+/-2 days						
Day	Within 60 days of Visit 2B	Up to 5 days prior to Visit 2B	Approx 30 day prior to Visit 3F	Day-10 to -4	Day -4 to -3	Day -2 to -1	Day 0	Day 1	Day 4	Day 8	Day 11	Day 15	Day19	Day 24
Informed Consent/Assent	X													
Eligibility check	X													
Demographic Data	X													
Medical History	X	X												
Physical examination	X	X		X										
Vital Signs	X			X			X[2]							
Weight (kg)	X	X			X									
Height (cm)	X													
Biochemistry sample[3]	X	X		X				X	X	X	X	X	X	X
Hematology sample[4]	X	X		X				X	X	X	X	X	X	X
PT or PTT/INR	X	X		X										
PB or BM cytogenetics[5]	X													
HIV-1/Hep B/Parvo B19	X													
Serum pregnancy test[6]	X	X		X			X							
Urinalysis[7]	X	X		X										
ECG	X													
Echocardiogram	X													
Chest X-ray[8]	X													
Pulse oxymetry	X			X										
Biopsy of skin lesions	X													
ADA-SCID diagnosis confirmation	X													
Questionnaire/QofL scale	X													
Infectious episodes	X			X										
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication[9]		X	X	X	X	X	X	X	X	X	X	X	X	X
Type and cross PRBC		X												
Bone marrow harvest			X											
PRBC transfusion			X											
Immune function[10]				X[11]										
PBMC banking for RCL				X[12]										

Study Period	**Screening**	**Pre-harvest Assessment**	**Bone Marrow Harvest**	**Baseline**	**Conditioning**		**OTL-101 Treatment**	**Short-term Follow-up**						
Visit number	1	2[1]	2	3[1]	3	3	3	3	3	3	3	3	3	3
Assessment number		A	B	C	D	E	F	G	H	I	J	K	L	M
Visit window								+/-2 days						
Day	Within 60 days of Visit 2B	Up to 5 days prior to Visit 2B	Approx 30 day prior to Visit 3F	Day-10 to -4	Day -4 to -3	Day -2 to -1	Day 0	Day 1	Day 4	Day 8	Day 11	Day 15	Day19	Day 24
Serum banking for RCL				X[12]										
Erythrocyte ADA enzymatic activity				X[12]										
Erythrocyte deoxyadenine nucleotide levels				X[12]										
Busulfan administration					X	X								
PK samples					X[13]	X[14]								
OTL-101 administration							X							

1. Subjects will be admitted to the hospital for Visits 2 and 3.
2. During the infusion of OTL-101 vital signs will be monitored according to standard institute protocols.
3. Biochemistry samples will include the assessment of parameters listed in Table 3. Albumin ALT, AST, ALP will be measured at all time points indicated in this table. Total bilirubin, total protein, total protein, creatinine, blood urea, sodium, potassium, chloride, and calcium will be measured at screening, baseline, Day 1 and all follow-up visits (Month 1 to Month 24). Magnesium and phosphate will be measured only at pre-harvest and baseline time points.
4. Hematology samples will include the assessment of parameters listed in Table 3 and will be measured at all time points indicated in this table.
5. If cytogenetic testing was not performed on cells from amniocentesis, assessment should be by karyotype, CGH, and WES
6. Serum pregnancy tests are required in females of child-bearing potential at screening, prior to bone marrow harvest, prior to conditioning (Busulfan) treatment, and before the infusion of OTL-101. Any positive test will immediately result in the withdrawal of the subject from the study and should be reported as per guidance in Section 8.2.4
7. Urinalysis will include the assessment of parameters listed in Table 3 and will be measured at all time points indicated in this table. Any abnormalities detected on dipstick at Screening, Pre-harvest and Baseline will require repeat dipstick and microscopic examinations.
8. If there is any evidence of severe lung disease on the screening chest X-ray, a CT scan is required in order to establish if the subject meets any of the specified pulmonary exclusion criteria defined in Section 4.2.
9. Standard post-transplant medication will be administered from Day 0 and recorded in the eCRF.
10. Immune function will be assessed absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells, TREC levels, TCR Vβ usage, lymphocyte response to PHA (if sufficient cells), and serum immunoglobulin (IgG, IgA, IgM).
11. The immune function tests required for Visit 3, Assessment C can be performed at any point after provision of consent/assent and prior to Busulfan administration in younger subjects if deemed appropriate by the Investigator.
12. These test can be performed at anytime following BM harvest (Visit 2: Assessment B) and prior to OTL-101 administration (Visit 3: Assessment F) in younger subjects if deemed appropriate by the Investigator.
13. PK blood samples are required at the end of Busulfan infusion, +1, 2, 4, 8, and 13 hours post-infusion (+/- 15 minutes). These values will be used to determine the second dose of Busulfan.
14. One PK blood sample is required immediately before the second dose of Busulfan is administered, and then at the end of Busulfan infusion, +1, 2, 4, 8, and 13 hours post-infusion (+/- 15 minutes).

Study Period	**Screening**	**Pre-harvest Assessment**	**Bone Marrow Harvest**	**Baseline**	**Conditioning**		**OTL-101 Treatment**	**Short-term Follow-up**						
Visit number	1	2[1]	2	3[1]	3	3	3	3	3	3	3	3	3	3
Assessment number		A	B	C	D	E	F	G	H	I	J	K	L	M
Visit window								+/-2 days						
Day	Within 60 days of Visit 2B	Up to 5 days prior to Visit 2B	Approx 30 day prior to Visit 3F	Day-10 to -4	Day -4 to -3	Day -2 to -1	Day 0	Day 1	Day 4	Day 8	Day 11	Day 15	Day19	Day 24

Abbreviations: ADA=adenosine deaminase, ALP=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartate aminotransferase, BM=bone marrow, CGH= Comparative Genome Hybridization, CT=computer tomography, ECG=electrocardiogram, eCRF=electronic case report form, HIV=human immunodeficiency virus, IgA=immunoglobulin A, IgG=immunoglobulin G, IgM=immunoglobulin M, INR=international normalized ratio, NK=natural killer, PB=peripheral blood, PBMC=peripheral blood mononuclear cells, PHA= Phytohemagglutinin, PK=pharmacokinetic, PRBC=packed red blood cells, PT=prothrombin, PTT=prothrombin time, QofL=quality of life, RCL= Replication Competent Lentivirus, SCID=severe combined immunodeficiency, TCR= T-cell receptor, TREC= T-cell receptor excision circle, WES= whole exome sequencing.

Table 2: Schedule of Events – Month 1 to Month 24

Follow-up Period	**Month 1 (Day 30)**	**Day 42[1]**	**Month 3**	**Month 6**	**Month 9**	**Month 12**	**Month 18**	**Month 24**	**ET**
Visit number	4	5	6	7	8	9	10	11	N/A
Visit window	+/- 1 week	+/- 1 week	+/- 2 weeks	+/- 2 weeks	+/- 2 weeks	+/- 4 weeks	+/- 4 weeks	+/- 4 weeks	
Physical examination	X		X	X	X	X	X	X	X
Vital Signs	X		X	X	X	X	X	X	X
Weight (kg)	X		X	X	X	X	X	X	X
Height (cm)	X		X	X	X	X	X	X	X
Biochemistry sample[2]	X	X	X	X	X	X	X	X	X
Hematology sample[3]	X	X	X	X	X	X	X	X	X
Questionnaire/QofL scale				X		X	X	X	X
Infectious episodes	X		X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X
Concomitant Medication[4]	X	X	X	X	X	X	X	X	X
Record IgG and PEG-ADA therapy received	X	X	X	X	X	X	X	X	X
Immune function[5,6]	X		X	X	X	X	X	X	X
Serum banking for RCL			X	X		X		X	X
Vector integration analysis			X	X		X	X	X	
Leukaemia assessment[7]				X		X	X	X	X
T-cell proliferation to PHA				X	X	X	X	X	X
Antibodies to tetanus toxoid						X	X	X	X
Presence of ADA gene in PBMC/granulocytes and/or lineage-sorted cells			X	X		X		X	X
RCL assay in PBMC			X	X		X		X	X
ADA enzymatic assay	X		X	X	X	X	X	X	X
Deoxyadenine nucleotides in RBC	X		X	X	X	X	X	X	X
Responder assessment[8]				X					

1. Day 42 assessment will use laboratory parameters to confirm if hematologic reconstitution has occurred. If this has not happened by Day 42 rescue medication, as per standard institute procedure, will be implemented. Any treatment administered to the subject will be recorded in the eCRF.
2. Biochemistry samples will include the assessment of parameters listed in Table 3. Albumin ALT, AST, ALP will be measured at all time points indicated in this table. Total bilirubin, total protein, total protein, creatinine, blood urea, sodium, potassium, chloride, and calcium will be measured at screening, baseline, Day 1 and all follow-up visits (Month 1 to Month 24). Magnesium and phosphate will be measured only at baseline and the conditioning time points.
3. Hematology samples will include the assessment of parameters listed in Table 3 and will be measured at all time points indicated in this table.
4. Standard post-transplant medication will be administered from Day 0 and recorded in the eCRF.

5. Immune function will be assessed by absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells, TREC levels, TCR Vβ usage, lymphocyte response to PHA (if sufficient cells), and serum immunoglobulin (IgG, IgA, IgM).
6. If IgG treatment is stopped, immunoglobulin levels will be required every month for the first 3 month after discontinuation. This is to ensure IgG levels hold before commencing vaccinations as per standard institute protocols.
7. A blood sample will be taken unless clinical/hematologic findings indicate potential leukoproliferation, whereby a bone marrow sample will be required. In addition to the time points specified in this table, this assessment should be performed if the nrLAM-PCR criteria is fulfilled (see Section 7.2).
8. Responder assessment includes: erythrocyte ADA enzyme activity, immune reconstitution, and gene-marked granulocytes as described in Section 2.2.1.

Abbreviations: ADA=adenosine deaminase, ALP=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartate aminotransferase, eCRF=electronic case report form, ET=early termination, IgA=immunoglobulin A, IgG=immunoglobulin G, IgM=immunoglobulin M, N/A=not applicable, NK=natural killer, PBMC=peripheral blood mononuclear cells, PCR=polymerase chain reaction, PEG= polyethylene-glycol, PHA= Phytohemagglutinin, QofL=quality of life, RBC=red blood cell, RCL= Replication Competent Lentivirus, TCR= T-cell receptor, TREC= T-cell receptor excision circle.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
ADA	Adenosine deaminase
AEs	Adverse Events
AIDS	Autoimmune deficiency syndrome
ALC	Absolute lymphocyte count
ANC	Absolute Neutrophil Count
AUC	Area under the curve
BM	Bone marrow
CA	Competent Authorities
CFR	Code of Federal Regulations
CFSE	Carboxyfluorescein succinimidyl ester
CGH	Comparative genome hybridization
CLIA	Clinical Laboratory Improvement Amendments
CMC	Chemistry, manufacturing, and control
CofA	Certificate of Analysis
CRO	Contract Research Organisation
CT	Computer Tomography
CTCAE	Common terminology criteria for adverse events
CSR	Clinical Study Report
d-AXP	Adenine deoxyribonucleotides
DFSP	Dermatofibro sarcoma protuberans
DMSO	Dimethylsulfoxide
DNA	Deoxynucleic acid
DSMB	Data safety monitoring board
ECG	Electrocardiogram
EDC	Electronic Data Capture
eCRF	Electronic Case Report Form
EFS	Elongation Factor 1α Short form
ERT	Enzyme replacement therapy
FACS	Fluorescence-activated cell sorting
FDA	Food and Drink Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GOSH	Great Ormond Street Hospital
GRV	Gammaretroviral vector
GTMP	Gene therapy medicinal product
GvHD	Graft versus host disease
Hb	hemaglobin
HCG	Human Chorionic Gonadotropin

HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
INR	International normalized ratio
IO	Insertional oncogenesis
IRB	Institutional Review Board
IVIM	Immortalization studies *in vitro*
LTR	Long terminal repeat
LV	Lentiviral vector
MedDRA	Medical Dictionary for regulatory Activities
MUD	Matched unrelated donor
NIAID	National Institute Allergy and Infectious Diseases
NK	Natural Killer
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PEG	Polyethylene-glycol
PHA	Phytohemagglutinin
PICC	Peripherally inserted central catheter
PJP	Pneumocystis jirovecii prophylaxis
PK	Pharmacokinetics
PRBC	Packed red blood cells
PT	Prothrombin
PTT	Partial thromboplastin time
ddPCR	Differential display polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RBC	Red Blood Cell(s)
RCL	Replication competent lentivirus
RT	Reverse transcriptase
SAE	Serious Adverse Event/Experience
SAP	Statistical analysis plan
SAS®	Statistical Analysis System®
SCID	Severe combined immunodeficiency
SIN	Self-inactivating
SMX	Sulfamethoxazole
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction

TCR	T-cell receptor
TMP	Trimethoprim
TREC	T-cell receptor excision circle
UCB	Umbilical cord blood
UCL	University College London
UCLA	University of California Los Angeles
UK	United Kingdom
ULN	Upper Limit of Normal Range
US	United States
VCN	Vector copy number
WES	Whole exome sequencing
WHO	World Health Organisation

1 BACKGROUND INFORMATION

1.1 Introduction

A brief overview of the disease and the protocol treatment (OTL-101) is provided in this section; however, a comprehensive review is supplied in the Investigator Brochure (IB).

1.1.1 Severe Combined Immunodeficiency due to Adenosine Deaminase Deficiency

Severe combined immunodeficiency (SCID) is a heterogeneous group of inherited disorders characterised by a profound reduction or absence of T lymphocyte function (Chinn 2015). Adenosine deaminase (ADA)-SCID was identified almost four decades ago and represents approximately 15-20% of all cases of SCID. Adenosine deaminase is a ubiquitous enzyme whose deficiency results in metabolic toxicity. The main features of the disease are impaired differentiation and function of T, B and natural killer (NK) lymphocytes, recurrent infections, and failure to thrive. In addition, non-immunological abnormalities occur as the consequence of the systemic metabolic defect, indicating that the disease is more complex than other forms of SCID. Adenosine deaminase-SCID is a genetic disorder and mutations in the *ADA gene* cause a lack or the poor functioning of the ADA enzyme leading to accumulation of Ado, d-Ado, and adenine deoxyribonucleotides (d-AXP) in plasma, red blood cells (RBC), and tissues. The severity of genetic mutations and the residual ADA activity are usually related to the age of onset, clinical manifestations, and levels of d-AXP (Hirschhorn 1990).

The majority of ADA-SCID patients are diagnosed in the first year of life. The patients rarely survive beyond 1 to 2 years unless immune function is restored, or contact with pathogens is avoided by creating a sterile environment around the patient (the so-called “bubble-children”). Lymphopenia, absence of both cellular and humoral functions, developmental delay, failure to thrive, and recurrent infections due to fungal, viral, and opportunistic agents are the key features of these early onset forms. These forms are essentially indistinguishable from other forms of SCID, with the exception for bony abnormalities in 50% of patients.

1.1.2 Treatment Options

Two therapeutic methods are currently used for the treatment of ADA SCID, hematopoietic stem cell transplantation (HSCT) and ADA enzyme replacement therapy (ERT) but both options have significant limitations. This paved the way for the proposed OTL-101 treatment.

1.1.2.1 Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation from allogeneic human leukocyte antigen (HLA)-compatible sibling donors is the treatment of choice for patients with SCID and other severe variants of primary immunodeficiency resulting in long-term survival of about 90% of patients and effective immune reconstitution (Ferrua 2010). However, as only 10–20% of patients are able to receive HSCT from an HLA-identical family member (Stephan 1993, Antoine 2003), transplants are often performed from mismatched family or matched unrelated donors (MUD). Despite improvements in pre-conditioning and post-transplant care, the use of less well matched HSCT is associated with reduced survival (Gaspar 2009, Hassan 2012) and delayed or suboptimal immune reconstitution in a significant fraction of surviving patients as a result of poor early engraftment or gradual decline in immune functions (Railey 2009). Complications such as Graft versus Host Disease (GvHD), autoimmune and inflammatory manifestations, persistent infections, and disease-related issues are often observed.

If a matched sibling/family donor is unavailable, most ADA-SCID patients are treated with ADA ERT. Enzyme replacement therapy leads to metabolic correction and clinical

improvement, but the immunological reconstitution is often incomplete and dysregulated (Hershfield 1987, Gaspar 2009, Sauer 2012) and therefore individuals remain at risk of severe infection. This approach does not represent a curative treatment modality and is not always available as a long-term option. Enzyme replacement therapy with polyethylene-glycol (PEG)-ADA was developed based on the concept that reduction of ADA substrate concentration in plasma results in lowering of intracellular concentrations of metabolites (Hershfield 1995). An intramuscular injection of PEG-ADA is administered weekly or bi-weekly, and is considered to be an excellent (although very expensive) short-term measure to stabilize children with ADA-SCID; however, prolonged use has associated risks as described in the IB.

1.1.2.2 Gene Therapy

Gene therapy offers an alternative treatment for ADA-SCID whereby a correctly functioning copy of the *ADA gene* is introduced into hematopoietic stem cells (HSCs) that have been harvested from the patients themselves using a gene transfer vector. The transduced cells are then returned to the patient where they initiate immune reconstitution much like HSCs from a healthy donor. Because the cells originate from the patient, the likelihood of complications is reduced and there is no need to find a suitable donor. Importantly, unlike ERT, the treatment is curative. Earlier approaches relied on murine gammaretroviral vectors (GRV) to introduce the *ADA gene* into the patient's cells. Lentiviral vectors (LV) are derived from components of the human immunodeficiency virus (HIV)-1 lentivirus and display several attributes that make them potentially more effective and safe than retroviral vectors (Naldini 1996, Zufferey 1998) as described in Section 1.4.2.

No gene therapy treatment has been approved in United States (US) to date. In Europe, Strimvelis® is approved for the "treatment of subjects with ADA-SCID, for whom no suitable HLA matched related stem cell donor is available". Strimvelis® is a GRV based ex vivo gene therapy treatment.

1.2 Name and Description of Protocol Treatment

The protocol treatment (OTL-101) is a cell suspension comprising autologous CD34+ hematopoietic stem cells that are transduced *ex vivo* with Elongation Factor 1α Short form (EFS) LV encoding for the human *ADA gene*.

To create OTL-101, autologous CD34+ HSCs from ADA-SCID subjects will be modified using EFS-ADA LV, a self-inactivating (SIN) LV expressing the *ADA gene* codon optimized for human use and regulated by EFS.

The cryopreserved formulation will be thawed at the subject's bedside and infused directly without any further manipulation.

Orphan Drug Designation and Breakthrough Therapy designation were granted for this gene therapy medicinal product (OTL-101) from the Food and Drug Administration (FDA) on 21 October 2014 and 17 August 2015, respectively.

1.3 Findings from Non-Clinical and Clinical Studies

1.3.1 Non-Clinical Studies

The non-clinical studies demonstrated the EFS-ADA LV developed by the Sponsor is able to transduce different immortalized cell lines as well as CD34+ cells (the target cells for human use) from healthy donors and ADA-SCID patients. Transduction efficiency was at levels comparable to GRV used successfully in gene therapy clinical studies. However, significant

higher levels of ADA expression were seen per vector copy in transduced cells from umbilical cord blood (UCB) or bone marrow (BM) of normal or ADA-deficient individuals. *In vivo*, CD34+ cells from ADA-SCID patient bone marrow were transduced with the EFS LV encoding for the human *ADA gene* before being injected in immunodeficient mice. The studies demonstrated that the transduced cells engrafted and differentiated in a human xenograft model. Finally, a proof-of-concept study was performed in an experimental ADA deficient mouse model. This study demonstrated that cells transduced with the EFS LV encoding for the human *ADA gene* enabled T and B cell recovery; in addition to ADA enzymatic activity. Overall these results demonstrate the biological activity of CD34+ cells transduced *ex vivo* with EFS-ADA LV.

1.3.2 Toxicology Studies

Toxicology studies were performed to assess safety of the OTL-101 product. Due to its nature, a particular focus was made on assessment of potential genotoxicity. A first round of studies demonstrated that the EFS LV (encoding the human *ADA gene*) is not mutagenic and did not induce tumors in *in vivo* models. In addition, in a separate study, the proliferative capacity of clones of cells transduced with the vector was assessed and was found to be negligible. Therefore, OTL-101 is considered to have a safe profile.

Overall these results demonstrate that the EFS LV (encoding the human *ADA gene* or a reporter *green fluorescent protein gene*) is not mutagenic and does not induce tumours in *in vivo* models.

1.3.3 Clinical Studies

Two parallel Phase I/II studies investigating the clinical effectiveness and safety of EFS-ADA LV-modified autologous CD34+ cells have been performed to date.

A first study, entitled: “Autologous Transplantation of Bone Marrow CD34+ Stem/Progenitor Cells after Addition of a Normal Human ADA cDNA by the EFS-ADA LV for Adenosine Deaminase-Severe Combined immunodeficiency”) at University of California Los Angeles (UCLA) in the US began in May 2013. Currently, 20 subjects have been treated under this protocol (as of 14 October 2016) and a single subject has been treated under an expanded access program.

A second study, conducted in parallel, entitled: “Phase I/II, non-controlled, open-label, non-randomised, single-centre study to assess the safety and efficacy of EFS-ADA LV mediated gene modification of autologous CD34+ cells from ADA-deficient individuals” began in August 2012 at Great Ormond Street Hospital (GOSH) University College London (UCL) in the United Kingdom (UK). Currently, 10 subjects have been treated under this protocol and a further 10 subjects have also been treated under compassionate use in the UK (as of 14 October 2016).

In both studies, subjects receive PEG-ADA ERT and Busulfan conditioning before receiving a fresh formulation of EFS-ADA LV-transduced CD34+ HSCs . Following treatment, subjects continue to receive PEG-ADA ERT for a further 30 days post OTL-101 infusion. This continuation of ERT during the peri-transplant period potentially maximizes engraftment of cells when the niche is low in cytotoxic substrates and minimizes the extent to which the subject becomes transiently more immune-deficient prior to the immune reconstitution induced by the gene therapy.

In both studies, subjects have received comparable doses, characterized by similar viability and percent CD34+ HSCs, as well as in transduction efficacy (vector copy number (VCN)) and

efficiency (vector positive colony forming unit analysis) in the OTL-101 product. The number of vector copies detected over time in peripheral blood cells after OTL-101 infusion is consistent across the two studies. Most importantly, there appears to be extremely consistent immune reconstitution outcomes between the two studies: cell counts for all of the T and B cell populations and subpopulations demonstrate consistent and stable immune reconstitution over time post GTMP infusion at 6, 12, and 24 months in both studies. As per 31 March 2016 data cut, the overall survival for the both studies is currently 100%. The event free survival for the second study (GOSH/UCL, UK) is currently 93.3%. One subject from the second study was clinically indicated to restart ERT with PEG-ADA, although none from the first study (UCLA) has required a restart of PEG-ADA (N=18, as per 31 March 2016 data cut).

No serious unexpected adverse events have been reported in both studies to date. There have been no vector-related complications and no instances of leukoproliferation, as reported in GRV studies for other immune deficiencies. There have been no cases of autoimmunity with the EFS-ADA LV reported to date. The safety profile of the two ongoing studies have been remarkable and the benefit/risk profile remains positive.

Since both studies are still ongoing, formal analysis comparing both studies has not been completed.

1.4 Known and Potential Risks and Benefits

1.4.1 Potential Benefits

Adenosine deaminase-SCID represents a paradigmatic approach for gene therapy for inherited blood borne disorders. The results of clinical studies from 2000 onwards indicate that gene therapy with non-myeloablative conditioning is associated with significant clinical clinical benefit and should be considered for all ADA-SCID patients lacking an HLA-identical sibling/family donor (Aiuti 2002, Aiuti 2009, Gaspar 2011, Candotti 2012). However, LV offer several potential advantages over murine GRV used in the previous studies, including the recently European Union approved treatment, Strimvelis®, GlaxoSmithKline. There is no gene therapy treatment for ADA-SCID approved in the US to date.

Lentiviral vectors lack dependence on cell division such that the culture period and exposure to cytokines can be minimized and HSC activity can theoretically be preserved.

Durable, life-long clinical benefit is highly dependent on the successful engraftment of genetically modified long term repopulating HSC. The improved correction of HSCs should result in improved immune recovery due to correction of both T and B cell populations.

Most importantly, LV have potential for a more favorable safety profile. Insertional oncogenesis (IO) is a possible consequence of the integration of retroviral vectors into the human genome, in which transcription of the therapeutic gene is under the control of the viral enhancer promoters present in the long terminal repeat (LTR). In previous GRV studies, oncogenesis has been caused by the *Moloney Murine Leukemia Virus gene* delivery vector integrating and transactiving an adjacent oncogene (Hacein-Bey-Abina 2008, Howe 2008, Braun 2014). Thus far, none of the ADA-SCID subjects treated with GRV (N=42) have had evidence of IO despite integration events in oncogenes associated with IO in other types of SCID patients treated with GRV genetically-modified autologous HSC. Nevertheless, the use of alternative vector systems such as the LV with the SIN LTR configuration have improved integration profiles (Biffi 2011) and allows for the use of safer, less transactivating cellular promoters. Immortalization studies *in vitro* (IVIM) have shown that the potential of SIN LV

with cellular promotors to transactivate neighboring genes is significantly diminished (Modlich 2006, Modlich 2009). Furthermore, the EFS cellular promoter has resulted in negligible outgrowth of clones in the IVIM assay (Zychlinski 2008, Carbonaro 2014).

Therefore the use of LV and EFS-ADA LV, in particular, can thus promote similar or even improved immune recovery with the potential for enhanced genetically-modified HSC activity and an enhanced safety, and therefore represents a significant advantage in furthering the use of gene therapy for ADA-SCID.

Transplants of autologous CD34+ HSCs transduced with the EFS-ADA LV have resulted in clinical benefits in the vast majority of subjects treated to date. It is too early to determine whether these subjects will have durable, life-long *ADA gene* expression/enzyme production and immune function, but the results indicate 100% survival, with all but one subject stopping PEG-ADA ERT. The longest follow-up is 58 months. The ideal outcome is for the treated subjects to develop improved immune function and not require either a rescue bone marrow transplant or further PEG-ADA ERT in the long-term.

The cryopreserved formulation of OTL-101 will be implemented within this protocol to improve the limitations of the fresh cell formulation, which only remains stable for 6 hours after manufacture. The rationale of this cryopreserved formulation is to allow the OTL-101 treatment to be administered at a local clinic, minimizing the travel burden on the subject and the family, to enable standardization of the Busulfan conditioning of subjects prior to dosing and to allow the infusion of a fully quality controlled tested and released OTL-101 product.

The aim of this protocol is to expand the safety and efficacy database for OTL-101 and support analytical chemistry, manufacturing, and control (CMC) comparability between fresh and cryopreserved OTL-101 formulations.

1.4.2 Potential Risks

1.4.2.1 Potential Risks Related to the Protocol Treatment

The major risks to be considered with OTL-101 treatment are:

1) Insertional oncogenesis (and leukemia) - risks related to IO have occurred during clinical studies of gene transfer using GRV in subjects with X-linked SCID and Wiskott Aldrich Syndrome, but not ADA-SCID thus far. Studies have demonstrated that the risks of insertional mutagenesis are lower with LV that lack strong LTR enhancer/promoter sequences (as in the EFS-ADA LV) than with GRV with an intact LTR. In current LV subjects (39 subjects treated to date), there have been no cases of oncogenicity. Pre-clinical *in vitro* insertional mutagenesis studies have confirmed that the LV used in this clinical program has a lower risk versus the GRV.

2) Replication-competent virus exposure - there is a small possibility and risk from this gene therapy treatment that undetected Replication Competent Lentivirus (RCL) might be present and cause an active HIV infection in the subjects. To date there has been no instance of RCL in subjects treated with autologous genetically modified HSC with a GRV or LV.

3) Germ-line transmission of vector sequences - as the EFS-ADA LV will be used to transduce *ex vivo* $CD34^+$ cells, rather than a direct administration to subjects via parenteral route, the probability of germ-line transmission is considered to be negligible.

4) Allergic/immunological responses to cell processing excipients - stem cell factor and thrombopoietin are used to maintain stem cell viability and to promote gene transfer during the *ex vivo* transduction process. When these factors are administered parenterally, they may have untoward immunologic or allergic consequences such as thrombocytopenia or allergic reactions.

5) Risk of using cryopreserved bone marrow stem cells. Although *in vitro* tests showed that cryopreserved genetically-modified bone marrow CD34+ HSC were similar to the fresh cells used in previous clinical studies, it is possible that the cryopreserved formulation of OTL-101 will not work as well in subjects, resulting in poorer recovery of immune function or even the need to receive back the unmodified bone marrow that was saved as back-up.

6) As thawed OTL-101 is infused, an unusual odor or bad taste in the mouth may be experienced. This odor or taste is from the substance dimethylsulfoxide (DMSO) used to protect the cells when OTL-101 is cryopreserved . Some subjects may cough or feel slightly nauseated or chilled when they receive thawed product containing DMSO. In rare cases, DMSO may cause side effects such as low blood pressure, fast heart rate, or shortness of breath. Subjects will be monitored closely during the OTL-101 infusion.

7) Immune response against “normal” ADA – as a result of "immunisation" to normal human ADA produced from the transgene. Most subjects will have previously received bovine PEG-ADA ERT, which is more likely to be immunogenic than human ADA. Also, development of significant inhibitory antibodies has been relatively rare in patients treated with PEG-ADA ERT alone.

8) Other immunological risks - onset of autoimmunity is of concern in post-treatment ADA-SCID patients.

9) Immune dysregulation - gene transfer research may result in only partial improvement of the immune system. While this is unlikely to be a problem, it is possible that partial improvement leading to an "imbalance" of the immune system could have adverse effects. Two children treated in the UCLA study using fresh OTL-101 formulation developed an inflammatory response with fevers and rashes requiring hospitalization about 4-12 months post OTL-101 infusion. This reaction may have been associated with the response to gene therapy treatment, with temporary immune dysregulation occurring as the immune system developed. Both children were treated with steroids and have fully recovered, suggesting the inflammatory reaction may be temporary. Both children remain free of PEG-ADA therapy.

10) Failure of the treatment.

These risks are fully described in the IB.

1.4.2.2 Potential Risks Related to Bone Marrow Harvesting

CD34+ cells will be isolated from BM. There is potential risk associated with the BM harvesting procedures, which entails general anaesthesia, multiple percutaneous aspirations from the posterior pelvic bone and a blood cell transfusion.

1.4.2.3 Potential Risks Related to Concomitant Therapy

Busulfan

Busulfan is used for conditioning prior to the re-infusion of the HSCs. The Busulfan dose to be used in this clinical study will target a specific area under the curve (AUC), which will ensure more precise dosing and may reduce the risks of toxicity. The risks attached to Busulfan are minimal, although there is a very small possibility of Busulfan-related cancer.

More common risks associated with Busulfan at higher doses include nausea, vomiting, diarrhoea, constipation, loss of appetite, mouth sores, stomach/abdominal pain, dizziness, swelling ankles/feet/hand, flushing, headache, trouble sleeping and a period of neutropaenia. However, the above systemic side-effects have not been seen at the proposed Busulfan dose for this study.

In the Sponsor's experience to date, there have been no Busulfan-related serious adverse events (SAEs).

Pegylated-ADA

The withdrawal of PEG-ADA, which is required 1 month after OTL-101 administration, can have adverse effects on immunity, as well as on other organ systems. It is possible that a very severe infection may develop during this time, which could be fatal if the subject does not respond to OTL-101 infusion.

In the Sponsor's experience to date, there have been no severe infections nor causalities, related to stopping PEG-ADA ERT, 1 month after OTL-101 infusion as per protocol.

1.4.2.4 Other Potential Risks

Participation in this clinical study may have deleterious effects on subsequent attempts at allogeneic HSCT due to the "immunization" to normal human ADA produced from the transgene. Gene therapy for ADA-SCID using autologous stem cells, albeit with other vectors, has now been performed in 79 subjects, with over 15 years of follow-up. The results show that these subjects did not experience any permanently debilitating nor deleterious side effects, suggesting that the risk of long term side effects is low.

As in any new form of therapy, there may be risks that are unknown or not anticipated.

1.5 Selection of Protocol Treatment Dosage

Cryopreserved autologous CD34+ HSCs transduced *ex vivo* with EFS LV encoding for the human *ADA gene* will be administered at a dose range of 2.0 x 10e6 to 20 x 10e6 CD34+ cells/kg. The selected dose is similar to the standard dose of allogeneic HSCT. One infusion of a target minimum of 2.0 x 10e6 CD34+ cells/kg of OTL-101 is deemed sufficient to restore ADA activity.

A more detailed description of OTL-101 administration is presented in Section 3.4 and Section 3.7.

1.6 Compliance Statement

The clinical study will be conducted in compliance with the requirements of independent ethics committees/institutional review boards (IEC/IRB), informed consent regulations, the Declaration of Helsinki and the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines. Any episodes of noncompliance will be documented. The electronic data capture (EDC) system will comply with the FDA, 21 Code of Federal

Regulations (CFR) Part 11, Electronic Records, Electronic Signatures, and FDA, Guidance for Industry: Computerized Systems Used in Clinical Trials. In addition, the study will adhere to all local regulatory requirements. Before initiating a clinical study, the Investigator/institution should have received a written and dated approval/favourable opinion from the IEC/IRB for the study protocol/amendment(s), written informed consent forms, any consent form updates, subject emergency study contact cards, subject recruitment procedures (e.g. advertisements), any written information to be provided to subjects and a statement from the IEC/IRB that they comply with GCP requirements. The IEC/IRB approval must identify the protocol version as well as the documents reviewed.

After IEC/IRB approval, changes will require a formal amendment. Once the clinical study has started, amendments should be made only in exceptional circumstances. Changes that do not affect subject safety or data integrity are classified as administrative changes and generally do not require ethical approval. If ethically relevant aspects are concerned, the IEC/IRB must be informed and, if necessary, approval sought prior to implementation. Ethical approval on administrative changes will be obtained if required by local/site IEC/IRB.

The Investigator is responsible for performing the clinical study in accordance with this protocol and the ICH and GCP guidelines and for collecting, recording, and reporting the data accurately and properly. Agreement of the Investigator to conduct and administer this clinical study in accordance with the protocol will be documented in separate study agreements with the Sponsor and other forms as required by national authorities.

The Investigator is responsible for ensuring the privacy, health, and welfare of the subjects during and after the clinical study and must ensure that trained personnel are immediately available in case of a medical emergency. The Investigator must be familiar with the background to, and requirements of, the study and with the characteristics and properties of OTL-101 as described in the IB.

1.7 Population to be Studied

The population to be studied in this protocol is ADA-SCID subjects who are aged ≥30 days to <18 years of age, lacking a medically eligible HLA-matched sibling donor.

Subjects should meet all of the inclusion criteria specified in Section 4.1 and none of the exclusion criteria defined in Section 4.2.

1.8 Relevant Literature and Data

Not applicable.

2 PURPOSE OF THE STUDY AND STUDY OBJECTIVES

2.1 Purpose of the Study

The primary purpose of the study is to further explore the efficacy and safety of gene therapy with OTL-101, as an alternative treatment option for ADA-SCID patients. The current treatment of choice for ADA-SCID patients is a HLA-matched sibling/family HSCT, however the number of patients eligible for this procedure ranges from 10 to 20% (Hassan 2012). OTL-101 is a gene therapy that aims to use genetically modified autologous CD34+ HSC from the individual patients to improve the outcome of transplantation to a wider population. Two ongoing Phase I/II studies use a fresh cell formulation that is required to be infused within 6 hours of manufacture. A cryopreserved formulation of OTL-101 was developed to enable the treatment, once manufactured from the individual subjects' CD34+ cells, to be frozen and stored until the OTL-101 product is fully released (i.e. all quality control testing performed), allowing the subject to receive targeted conditioning prior to transplantation. The cryopreserved formulation will also permit the treatment to be shipped and delivered to a local expert site, thereby reducing the travel burden on the recipient and their family. The advantages of a cryopreserved formulation are not limited to shelf-life and include; an improved manufacturing process; improved quality of the OTL-101 product, thus allowing for all quality control tests to be performed and to permit standardisation of preconditioning regimens prior to OTL-101 infusion. Three subjects have already been treated with cryopreserved cells (with a different cryoprotectant as the one used under this protocol); two patients treated under Compassionate Use Program and one patient treated as an exemption in the US. All three subjects demonstrated immune reconstitution and are currently event free indicating success of the OTL-101 treatment.

Additionally, the purpose of this study is to support the analytical CMC comparability of the cryopreserved versus fresh OTL-101 formulation with an interim analysis of clinical outcomes from the five subjects, based on predictive parameters at 6 months of overall survival and event free survival: gene-marked granulocytes, ADA enzyme activity, and absolute $CD3^+$ cell number.

These parameters are deemed adequate predictors at 6 months as well as long-term predictors for overall survival and event free survival based on the sponsor experience and previous gene therapy treatment.

- Gene- marked granulocytes

  After genetic modification of autologous CD34+ hematopoietic stem cells (HSCs) and reinfusion to the subject, the presence of genetically modified cells both in the periphery and in the BM can be used as a bona fide marker of the existence and the persistence of transduced cells in the subject. Genetically modified cells engraftment is usually assessed in the blood compartment that is readily accessible, via evaluation of the pro-viral VCN by qPCR in various blood cell populations (especially the granulocytes). Thus, by measuring the VCN in granulocytes we are able to assess the correction of long-lived genetically modified progenitors in the BM (as VCN in BM CD34+ cells shows a close positive correlation with VCN in granulocytes). In our ongoing studies with fresh product formulation, over 3 months after administration of OTL-101 the granulocyte VCN rises and is then stable up to 2 years after gene therapy treatment (based on our current follow up), therefore if a plateau is reached at 6 months (above

1/10,000 cells), it is predictive of sustained effect. These data are similar between both ongoing Phase I/II studies.

- ADA Enzyme Activity in red blood cells

  ADA enzyme activity in RBC reflects normalized/corrected biological activity of transduced CD34+ HSCs. As per experience from fresh formulation studies, there is peak observed at 3 months post OTL-101 infusion and then the level stabilizes for up to 24 months, based on our current data. Therefore, erythrocyte ADA enzyme activity above baseline/pre-treatment level compared to post treatment level at 6 months is predictive of long-term efficacy.

- CD3+ cells number

  The engraftment of genetically modified CD34+ HSCs in this disease setting should give rise to immune system development. Based on experience from previous clinical trials based on gene transfer to CD34+ HSCs, longitudinal copy number quantitative determination in T cells revealed that the T cell pattern was stable with time. It is important to remind that T cell normal values were reached between 2 to 5 months after OTL-101 infusion in subjects. Therefore, corrective levels of T cells were stable from 6 months to 10 years after OTL-101 infusion. Data suggests that once engraftment of genetically modified HSCs occurs and immune development is seen, CD3+ cells >200/mm^3, there is no diminution of immune cell development (Hacein-Bey-Abina 2010). The data from the clinical studies using the fresh formulation of OTL-101 concur with these findings. Clinically, this also means that once immune recovery is seen and is sustained, the need to restart ERT is negated.

2.2 Study Objectives

2.2.1 Primary Study Objectives

There are two primary objectives for this study aimed to determine;

1. The success of treatment at the subject level ("responder analysis") 6 months post OTL-101 infusion, defined as:

   a) Evidence of erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units),

   b) Evidence of immune reconstitution (absolute number of CD3 cells ≥200/mm^3),

   c) Detectable gene-marked granulocytes by differential polymerase chain reaction (dPCR)/qPCR (≥1/10,000 cells).

   This 6 month data will be used to compare the proportion of responders among subjects treated with the cryopreserved population with that in a comparable population obtained from one of the ongoing Phase I/II studies using the fresh formulation.

2. The overall survival and event free survival at 12 months among ADA-SCID subjects treated with a cryopreserved formulation of OTL-101.

   Overall survival is defined as the proportion of subjects alive. Event-free survival is defined as the proportion of subjects alive with no "event", an "event" being the resumption of PEG-ADA ERT or the need for a rescue allogenic HSCT, or death.

2.2.2 Secondary Study Objectives

The secondary objectives to be evaluated among ADA-SCID subjects treated with a cryopreserved formulation of OTL-101 include confirmation of the overall survival and event free survival at 24 months, safety evaluation including infection rates, quality of life and immune response.

2.2.3 Exploratory Study Objectives

Exploratory objectives include the measurement of biological correlates of efficacy including; percentage of gene marking in peripheral blood granulocytes, vector integration analysis, quantification of clonal diversity of vector integrants, T-cell receptor excision circle (TREC) and fluorescence-activated cell sorting (FACS) for T-cell receptor (TCR) V-β family use, ADA enzyme activity in erythrocytes, total adenine nucleotides in erythrocytes, and immune reconstitution.

3 STUDY DESIGN

3.1 General Design and Study Scheme

This is a prospective, non-randomized, single-cohort, longitudinal, single-center, clinical study designed to assess the efficacy and safety of OTL-101 cryopreserved formulation administered in ADA-SCID subjects aged between ≥30 days to <18 years of age, who are not eligible for an HLA-matched sibling/family donor and meeting the inclusion/exclusion criteria. This study aims to recruit 10 evaluable subjects.

The aim of this clinical study is also to assess the success of treatment at the subject level ("responder analysis") 6 months post OTL-101 infusion, using predictive criteria for overall survival and event free survival and to compare this with data obtained from one of the ongoing Phase I/II clinical studies using the fresh formulation of OTL-101.

Once informed consent has been obtained, the subjects enrolled will be screened to determine their full eligibility for participation over 1 month.

Eligible subjects will be hospitalized to undergo the harvesting of autologous CD34+ cells. A backup harvest of non-transduced CD34+cells will be obtained during the harvesting procedure to be used in the event of i) product damage during the thawing of OTL-101 that would prevent infusion of the product although the patient has already been conditioned or ii) lack of engraftment/hematopoietic reconstitution 42 days post infusion of OTL-101. A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of OTL-101 infusion.

OTL-101 is a cryopreserved formulation of autologous CD34+ hematopoietic stem cells transduced *ex vivo* with EFS LV encoding for the human *ADA gene* (OTL-101). To enable the release of the OTL-101 product for infusion, the cryopreserved OTL-101 product must meet various quality control criteria for safety, identity, viability, purity and potency. If OTL-101 meets the acceptance criteria and is released, the subjects will be readmitted for conditioning with Busulfan. The OTL-101 product will be infused after a minimal interval of at least 24 hours following the completion of Busulfan administration. Subjects may remain as an in-patient following autologous transplantation for approximately three to 35 days.

For subjects who have successfully received the OTL-101 product, PEG-ADA ERT use will be evaluated at the 1 Month follow-up visit (30 days post treatment ± 7 days) and discontinued at Day 30 (-3/+15) after the transplant. Hematologic reconstitution will be assessed at Day 42 (± 7 days), and in the event of no reconstitution the backup HSC sample will be administered, if the investigator believes this is in the subject's best interest. The subjects will then be seen at regular intervals to review their history, perform examinations and draw blood samples at Months 3, 6 and 9 (all ± 2 weeks) as well as Months 12, 18 and 24 (all ± 4 weeks). Any medically-indicated interventions, including the need to reinstate PEG-ADA ERT, will be assessed at all follow-up visits after Month 1. Hematopoietic reconstitution will be assessed again at the Month 6 visit and if there is still no evidence of cellular recovery, the subject will be given ERT and/or HSCT, if available and deemed appropriate by the Investigator. After the Month 24 visit, the subjects will have completed the study and may enter a long term registry.

This study will include three statistical analyses:

1. The first analysis will compare the success/failure data 6-months post OTL-101 infusion between fresh and cryopreserved OTL-101 formulations. The success/failure of OTL-101 will be defined by the three critera listed in the primary objectives (Section 2.2.1). This data will be compared with available data from one of the ongoing Phase I/II studies using the OTL-101 fresh formulation. This will support the CMC comparability data between OTL-101 cryopreserved and fresh formulations. Secondary and exploratory endpoints will also be described.
2. The second analysis will determine the 12 month overall survival and event free survival for all subjects. Secondary and exploratory endpoints, as well as baseline characteristics, subject disposition, and safety data will also be described.
3. The third and final analysis will be performed to determine the overall survival and event free survival for all subjects 24 months post OTL-101 infusion. Secondary and exploratory endpoints, as well as baseline characteristics, subject disposition, safety and Busulfan pharmacokinetic (PK) data will also be described.

Efficacy analyses will be primarily descriptive in nature.

The study will be overseen by an independent Data Safety Monitoring Board (DSMB), who will review the nature and severity of emerging safety data during the study. The DSMB will also liaise with the Principal Investigator directly to ensure the safety of participants during the study. The Investigator, subjects and their families will be informed about any significant emerging safety data.

The study schema is provided in Figure 1 below.

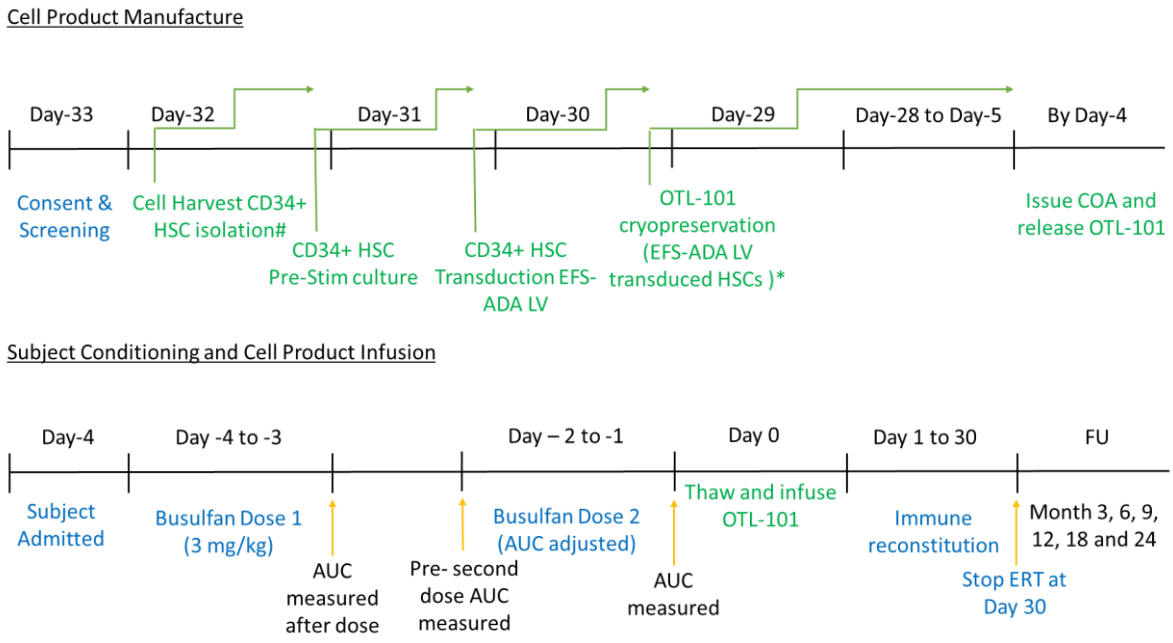

Figure 1: Study Scheme

Backup sample will be taken in the event of product damage or failure to achieve hematopoietic reconstitution.
* The timeframe for the cryopreservation process may vary.
Abbreviations: ADA= Adenosine deaminase, AUC = area under the curve, BM = bone marrowm, EFS = Elongation Factor 1α Short form, ERT = enzyme replacement therapy, LV = lentivirus vector.

3.2 Primary and Secondary Evaluations and Endpoints

3.2.1 Primary Efficacy Evaluations and Endpoints

The primary efficacy endpoints for this study include:

1. Evaluation of OTL-101 at 6 months, post OTL-101 infusion, in support of CMC assessing comparability of cryopreserved and fresh formulations, by evaluating the success of therapy for each subject based on all three parameters listed below and their thresholds being met:

   a) Erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units),

   b) Absolute CD3+ T-cell counts ≥200/mm^3, and

   c) Peripheral blood samples positive for vector sequences by qPCR(≥1/10,000 cells).

   Please note:Subjects not meeting any one criterion will be designated a failure (non-responder). Further details are described in Section 3.6.1.

   This data will be compared with subject data from the Phase I/II study using the fresh formulation.

2. Evaluate the overall survival and event free survival 12 months post OTL-101 infusion. Overall survival is defined as the proportion of subjects alive. Event-free survival is defined as the proportion of subjects alive with no "event", an "event" being the resumption of PEG-ADA ERT or the need for a rescue allogenic HSCT, or death.

Full descriptions of the efficacy assessments are presented in Section 7.1.

3.2.2 Secondary Efficacy Evaluations and Endpoints

Secondary efficacy endpoints include:

- Evaluation of the overall survival and event free survival 24 months post OTL-101 infusion.
- Use of immunoglobulin replacement therapies prior to and after OTL-101 infusion.
- Performance outcomes and quality of life measured by the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance and ability to practice sports, respectively.
- Rates of severe infections/opportunistic infectious episodes, defined as infections or severe infections requiring hospitalization or prolonging hospitalization and/or documented infections by opportunistic pathogens (e.g. interstitial pneumonia, intractable diarrhoea).
- Response to tetanus vaccination.
- Immune reconstitution: T and B cell reconstitution.

Full descriptions of the efficacy assessments are presented in Section 7.1.2.

3.2.3 Safety Evaluations and Endpoints

The safety and tolerability of OTL-101 will be assessed throughout the study by evaluating adverse events (AEs), clinical laboratory test results, vital signs measurements, electrocardiogram (ECG), physical examination results, and concomitant medication usage. In

addition, the safety of OTL-101 will be assessed through monitoring infections and any emergence of replication competent lentivirus, monoclonal expansion or leukemia due to chosen vector.

Full descriptions of the safety assessments are presented in Section 8.

3.2.4 Exploratory Endpoints and Evaluations

The exploratory endpoints will include laboratory correlates of efficacy that will be used to assess the level of gene correction, engraftment and immune reconstitution:

- Quantification of clonal diversity of vector integrants.
- TREC and FACS for TCR V-beta family use.
- Percentage gene marking in peripheral blood cells.
- Adenosine deaminase enzyme activity in erythrocytes.
- Total adenine nucleotides in erythrocytes.
- Vector integration analysis.
- Immune reconstitution
    - Absolute Lymphocyte Count.
    - Absolute numbers of T, B, NK lymphocytes in peripheral blood.
    - T lymphocyte proliferative responses to mitogen (PHA) and to antigens (tetanus toxoid after vaccination)
    - Serum immunoglobulin levels (IgG, IgA and IgM).

3.3 Randomization and Blinding

This is a non-randomised study and there is no blinding.

3.4 Protocol Treatment and Dosage

OTL-101 comprises autologous, $CD34^+$ HSCs transduced *ex vivo* with EFS-ADA LV, formulated and cryopreserved in CryoStor® CS5. The OTL-101 product will be manufactured using HSCs purified from the subject's BM. To enable its release for administration, the OTL-101 product must meet the acceptance criteria for a series of Quality Control assays of safety, identity, viability, purity and potency.

The dose of genetically-modified cells comprises a single administration of 2.0 to 20 x 10e6 CD34+ cells/kg.

A detailed description of the procedures for the administration of the OTL-101 product is provided in Section 3.7.

3.4.1 Protocol Treatment

OTL-101 is manufactured under Good Manufacturing Practice (GMP) guidelines, using the subject's own $CD34^+$ HSCs. OTL-101 is formulated in Cryostor CS5, filled into cryobags, cryopreserved and then stored in the vapor phase of liquid nitrogen (LN_2). Final product dose and infusion volume calculations are described in Section 6.1.1 and Section 17: Appendix 1. On the day the subject is to be infused, the cryopreserved OTL-101 product bag(s) will be transported to the bedside in a dry nitrogen shipping container. Immediately prior to infusion,

the bag(s) will be removed from the shipping container and thawed using either a plasma thawing device or a pre-warmed water bath (using sterile water). For thawing, the OTL-101 product bag(s) will be placed in an outer bag sleeve in case of a leak. After thawing, the OTL-101 product bag(s) will be attached to a sterile intravenous line fitted with a three-way stopcock and sterile syringe. The cells will be pulled into the syringe from the bag(s), the stopcock will be turned and then the contents of the syringe will be pushed through the intravenous line into the recipient. The OTL-101 product will be labeled as the Study Agent. Please note: when more than one bag is required to be infused, only one OTL-101 product bag per hour will be administered (Section 6.1.1).

3.5 Duration of Subject Participation

The study will consist of the following periods:

- Screening: approximately 2 months
- Bone marrow harvest of autologous cells: 1 day
- Manufacture and release of OTL-101: 30-45 days
- Pre-conditioning prior to transplant: 4 days
- OTL-101 administration: this may entail up to 4-6 weeks of in-patient stay, either at UCLA or the subject may be transferred from UCLA to a home hospital at 3-7 days post-transplant for the pursuit of subsequent hospitalization for up to 4-6 weeks.
- Post treatment follow-up: 2 years after the administration of OTL-101.

The total study duration for each subject is estimated to be up to 2 years and 4 months.

The study will start when the first subject has signed the informed consent form. Each subject's participation in the study will end at the time of his/her last visit, or death. The study will have ended after the last subject has completed the last study visit at 24 months post OTL-101 infusion, however the primary endpoints for this study will be overall survival and event free survival at 12 months post OTL-101 infusion.

On completion of their participation in this study, subjects will be invited and encouraged to participate in a registry for long term data collection according to guideline recommendations.

3.6 Stopping Rules and Discontinuation Criteria

3.6.1 Subject Stopping Rules and Criteria for Discontinuation

If any one of the following instances occur, all new enrollments to the protocol or the treatment of subjects will be halted, pending investigation of the cause and discussion with the DSMB for final recommendation/decision:

1. If there is one death or two Grade 4 treatment-related toxicities (see Section 8.2.1.1) for severity definitions). Please note Grade 4 Busulfan-related hematologic AEs such as transient leukopenia, anemia, thrombocytopenia that are anticipated, and have resolved within 42 days of transplant will not be considered a stopping rule.
2. If two subjects experience prolonged unresponsive pancytopenia, defined as an initial failure of hematologic reconstitution which does not improve following the administration of the subject's autologous back-up cells. A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three

independent and consecutive determinations over at least ten days after day 42 from the day of OTL-101 infusion.

If, at Day 90 following the initial infusion of OTL-101, the failure of hematologic reconstitution persists after the subject's autologous back-up cells have been infused, the subject can be said to be experiencing prolonged unresponsive pancytopenia. The subject should then receive standard care for pancytopenia.

3. If RCL is detected, and confirmed in one subject.
4. If a subject in the study develops hematological proliferative, monoclonal expansion or malignant disease (excluding dermatofibrosarcoma protuberans (DFSP)). A thorough investigation of the cause of the proliferation, expansion or malignancy, including proviral integration analysis, will be carried out.
5. If there are three non-responders, as defined in the primary endpoint criteria (Section 3.2.1), who will be designated an OTL-101 treatment failure and will be withdrawn from the study to permit further treatment with PEG-ADA ERT, or HSCT if available.

Any such finding will be discussed with the Sponsor and DSMB, and an agreement should be made and documented to detail the conditions under which the study can be resumed before enrolling the next subject. Evaluations of the study endpoints of subjects already enrolled and who have received OTL-101, will continue.

A subject may discontinue participation in the study at any time for any reason (for example lack of efficacy, withdrawal of consent, AE). The Investigator and/or Sponsor can withdraw a subject from the study at any time for any reason (for example protocol violation or deviation as defined in Section 4.3, non-compliance with the protocol, or AE).

The Investigator should assess the ongoing risk-benefit ratio of each subject during their participation in the study, in consideration of any AEs and the potential benefits of continued treatment with OTL-101 or any alternative therapies.

3.6.2 Early Study Termination

There are no formal rules for early termination of this study. During the conduct of the study, SAEs will be reviewed (see Section 8.2.1.) as they are reported from the study centre(s) to identify safety concerns.

The Sponsor may terminate this study at any time. Reasons for termination may include but are not limited to, the following:

- The incidence or severity of AEs in this or other relevant studies point to a potential health hazard for study subjects,
- Insufficient subject enrolment,
- Any information becoming available during the study that substantially changes the expected benefit risk profile of the study treatments.

3.7 OTL-101 Storage, Preparation, Administration, and Accountability

3.7.1 OTL-101 Storage and Security

OTL-101 is manufactured from the individual subject's bone marrow $CD34^+$ HSCs. The Subject's BM will be harvested at the clinical site and then transferred to a GMP compliant manufacturing facility for onward processing. At the GMP facility $CD34^+$ cells will be isolated, transduced, formulated, filled into cryobags and then cryopreserved. The autologous, cryopreserved OTL-101 treatment will be stored as part of a controlled inventory at ≤-135°C in the vapor phase of LN_2 until released for administration to the subject. The storage of OTL-101 will be in accordance with UCLA standard procedures. Access to OTL-101 will only be permitted for individuals designated by the Investigator who are responsible and trained staff listed in the site personnel log at the clinical site. For release, the product must meet defined acceptance criteria for Quality Control assays of safety, identity, viability, purity and potency. During the first harvest, a backup sample of non-transduced CD34+ cells will be retained in i) the event of product damage or below product release specifications of the OTL-101 that would prevent the infusion taking place even though the patient has already been conditioned or ii) lack of engraftment/hematopoietic reconstitution at 42 days post OTL-101 infusion (see Section 3.4). In the event that a second BM harvest is required, the same storage and security procedures will be followed. A second backup sample is only required if the first backup is not reliable, e.g. if contamination of the initial marrow harvest had occurred.

3.7.2 OTL-101 Production

OTL-101 is manufactured using autologous $CD34^+$ HSC purified from BM as the starting material. Following receipt of the subjects BM from the clinical site, OTL-101 will be manufactured at the GMP facility according to the following process:

1. On Day 1:
    i. $CD34^+$ HSC from the BM harvest are purified by immunomagnetic selection.
    ii. The $CD34^+$ HSC are placed into a pre-stimulation culture to initiate the OTL-101 manufacturing process.

    NOTE: On Day 1, a back-up fraction of mononuclear cells will be cryopreserved. This back-up will constitute ≥3.0 x 10e7 mononuclear cells/kg.
2. On Day 2:
    i. The $CD34^+$ HSC are placed in co-culture with the EFS-ADA LV to begin transduction.
3. On Day 3:
    i. The transduced cells are harvested, washed, formulated into Cryostor® CS5, and filled into cryobags.
    ii. The cryobags are cryopreserved using a controlled rate freezer.
    iii. Following completion of the controlled rate freezing process, the cryobags are stored in the vapor phase of LN_2 at ≤-135°C.

3.7.3 OTL-101 Administration

The clinical site will inform the GMP facility when the subject will require the OTL-101 treatment to be delivered. OTL-101 will be transported to the clinical site in a dry nitrogen shipping container at least 24 hours prior to the start of Busulfan administration. The OTL-101 product will be labeled as the Study Agent.

Following the receipt of the shipping container from the GMP facility, responsible and trained staff (listed in the site personnel log at the clinical site) will document receipt and follow the instructions below and as described in Section 17: Appendix 1:

- Evaluate the outer packaging for damage,
- Verify the paperwork attached to the exterior of the package (including the certificate of analysis [CofA]),
- Hold the shipping container in secure storage at ≤-135°C , according to UCLA standard procedures, until requested by the Investigator. Storage instructions are specified in Section 3.7.1.

Immediately prior to administration, clinical site staff will:

- Cross-check the subject's details against the paperwork accompanying the shipping container,
- Open the container and retrieve the temperature monitor (if the lid does not have a visible readout) and ensure that no out-of-temperature alarms have been triggered.

When the team is ready to infuse OTL-101, the cryobag(s) will be removed from the shipping container and the team will immediately ensure:

- A visual inspection of integrity,
- Verify (by two individuals) that the label is correct and confirm that the cryobag is intended for the particular subject.

Final product dose and infusion volume calculations are specified in Section 6.1.1. The cryobag(s) will then be thawed using either a plasma thawing device or a pre-warmed water bath (using sterile water). For thawing, the OTL-101 product bag will be placed in an outer bag sleeve in case of any fracture.

When thawed, the OTL-101 product bag will be attached to a sterile intravenous line containing a three-way stopcock and sterile syringe. The contents will be pulled into the syringe from the bag, the stopcock will be turned and then the contents of the syringe will be infused intravenously into the subject’s peripherally inserted central catheter (PICC) line or central venous catheter. Please note: when more than one bag is required to be infused, only one OTL-101 product bag per hour will be administered (Section 6.1.1). If more than one bag is needed to achieve the dose, the weight of the subject must be higher than 4 kg to comply with recommended limit of DMSO per kg body weight per day prior to infusion.

If any circumstance occurs which prohibits delivery of the OTL-101 treatment to the subject, details on how to return OTL-101 to the GMP facility are specified in Section 17: Appendix 1.

3.7.4 OTL-101 Accountability

OTL-101 is manufactured on an individual basis for subject-specific use, and all steps for manufacturing, storage, final preparation, release and transport are documented by GMP facility staff. A specific form for the recording of the product traceability can be found in Section 17, Appendix 1 (Global Investigational Product Accountability Log). All of the product thus manufactured will be administered to the subject on one occasion only. The OTL-101 treatment must be administered by a Investigator or designated clinician/registered nurse (who is listed on the site personnel log) and must be documented in the subject notes and the clinical care unit records.

When handling OTL-101 at the clinical site, the Investigator or designated individual (who is listed on the site personnel log) must follow the instructions in Section 17: Appendix 1. The Investigator, or designated individual, must maintain an inventory of all OTL-101 product received from the GMP facility, administered or dispensed to the subject, returned and destroyed. The OTL-101 treatmentmust not be infused to any person who is not the biological owner of the stem cells.

On request, or at completion or termination of the study, all prepared and unused OTL-101 must be returned to the GMP facility for destruction (unopened cryobags). Bags opened and partially used will be discarded at the clinical site. Written authorization from the Sponsor must be obtained prior to the destruction of any OTL-101 productat the clinical site.

Labeling of the OTL-101 product will comply with current GMP guidelines and local regulatory requirements.

It is the Investigator/institution's responsibility to establish a system for handling the OTL-101 productso as to ensure that:

- Deliveries of such products are correctly received by a study designated person (e.g., a pharmacist or study coordinator),
- Deliveries are verified and documented on the appropriate OTL-101 accountability form(s),
- Certificates of delivery and return are signed, by the Investigator or the designate, and copies retained,
- The OTL-101 productis handled and stored safely and properly,
- OTL-101 is only administered by trained and designated staff,
- The OTL-101 productis only administered to study subjects in accordance with the protocol requirements.

3.8 Maintenance of Randomisation and Blinding

This is a non-randomised study with no blinding.

3.9 Source Data Recorded on the Case Report Form

All data will be recorded in the electronic case report form (eCRF). As required by GCP, the Sponsor assigned monitor will verify, by direct reference to the source documents, that the data required by the protocol are accurately reported in the eCRF. The source documents must, as a minimum, contain a statement that the subject is included in a clinical study, the date that informed consent was obtained prior to participation in the study, the identity of the study,

diagnosis and eligibility criteria, visit dates (with subject status), OTL-101 administration, and any AEs and associated concomitant medications.

The items recorded directly in the eCRF and considered as source data must be described at each site in a site-specific document signed by the Investigator and filed in the Investigator site file. For this study the questionnaire would be considered source data entered directly in the study records.

The definitions of source data and source documents are given below:

Source Data: all original records and certified copies of original records of clinical findings, observations, or other actions necessary for reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies) and should include demography, medical history, physical examination, vital signs, evolution of clinical picture, AEs start and stop dates, and withdrawal of a subject and reason.

Source Documents: Original documents, data and records (for example hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilms or magnetic media, X-rays, subject files, and records kept by the pharmacy, laboratories and medicotechnical departments involved in the clinical study).

The subject must have consented to their medical records being viewed by the Sponsor's authorized personnel/representative, and by local, and possibly foreign, Competent Authorities (CAs). This information is included in the informed consent form.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Inclusion Criteria

All subjects must fulfill the following criteria to be included in the study:

1. Provision of written informed consent prior to any study related procedures. In this study consent must be provided by the parents/legal guardians and, where applicable according to local laws, a signed assent from the child,
2. Subjects ≥30 days and <18 years of age,
3. With a diagnosis of ADA-SCID based on:

   Evidence of ADA deficiency, defined as:

   i. Decreased ADA enzymatic activity in erythrocytes, leukocytes, skin fibroblasts, or in cultured fetal cells to levels consistent with ADA-SCID as determined by the reference laboratory, or

   ii. Identified mutations in ADA alleles consistent with a severe reduction in ADA activity,

   Evidence of ADA-SCID based on either:

   i. Family history of a first order relative with ADA deficiency and clinical and laboratory evidence of severe immunologic deficiency, or

   ii. Evidence of severe immunologic deficiency in subjects prior to the institution of immune restorative therapy, based on

   - Lymphopenia (absolute lymphocyte count (ALC) <400 cells/μL) OR absence or low number of T-cells (absolute CD3+ count < 300 cells/μL), or
   - Severely decreased T lymphocyte blastogenic responses to phytohemagglutinin (either <10% of lower limit of normal controls for the diagnostic laboratory, or <10% of the response of the normal control of the day, or stimulation index <10), or
   - Identification of SCID by neonatal screening revealing low TREC levels.

4. Ineligible for matched family allogeneic BM transplantation, defined as the absence of a medically eligible HLA-identical sibling or family donor, with normal immune function, who could serve as an allogeneic bone marrow donor.
5. Females of child-bearing age will be required to provide a negative pregnancy test 30 days prior to Visit 2.
6. Subjects and their parents/legal guardians must be willing and able to comply with study restrictions and to remain at the clinic for the required duration during the study period and willing to return to the clinic for the follow up evaluation as specified in the protocol.

4.2 Exclusion Criteria

Subjects will not be eligible for the study if any of the following criteria are met:

1. Ineligible for autologous HSCT as per clinical site criteria.

2. Other conditions which in the opinion of the Principal Investigator and/or Co-Investigators, contraindicate the harvest of bone marrow, the administration of Busulfan and the infusion of transduced cells, or which indicate an inability of the subject or subject's parent/legal guardian to comply with the protocol.
3. Hematologic abnormality, defined as:
   - Anemia (Hb <8.0 g/dl).
   - Neutropenia (ANC <500/mm^3). Note: ANC <500 with absence of myelodysplastic syndrome on bone marrow aspirate and biopsy and normal marrow cytogenetics are acceptable for eligibility.
   - Thrombocytopenia (platelet count <50,000/mm^3, at any age).
   - Prothrombin time or international normalized ratio (INR) and partial thromboplastin time (PTT) >2 x upper limit of normal (ULN) (subjects with a correctable deficiency controlled on medication will not be excluded).
   - Cytogenetic abnormalities on peripheral blood or bone marrow or amniotic fluid (if available).
   - Prior allogeneic HSCT with cytoreductive conditioning.
4. Pulmonary abnormality, defined as:
   - Resting O_2 saturation by pulse oximetry <90% on room air.
   - Chest X-ray indicating active or progressive pulmonary disease. Note: Chest X-ray indicating residual signs of treated pneumonitis is acceptable for eligibility.
5. Cardiac abnormality, defined as:
   - Abnormal ECG indicating cardiac pathology.
   - Uncorrected congenital cardiac malformation with clinical symptoms.
   - Active cardiac disease, including clinical evidence of congestive heart failure, cyanosis, hypotension.
   - Poor cardiac function as evidenced by left ventricular ejection fraction <40% on echocardiogram.
6. Neurologic abnormality, defined as:
   - Significant neurologic abnormality revealed by examination.
   - Uncontrolled seizure disorder.
7. Renal abnormality, defined as:
   - Renal insufficiency: serum creatinine ≥1.2 mg/dl (106 µmol/L), or ≥3+ proteinuria.
   - Abnormal serum sodium, potassium, calcium, magnesium or phosphate levels at >2 x ULN.
8. Hepatic/gastrointestinal abnormality, defined as:

- Serum transaminases >5 x ULN.
- Serum bilirubin >2 x ULN.
- Serum glucose >1.5 x ULN.

9. Oncologic disease, defined as:
   - Evidence of active malignant disease other than DFSP[2].
   - Evidence of DFSP expected to require anti-neoplastic therapy within the 5 years following the infusion of genetically corrected cells (if anti-neoplastic therapy has been completed, a subject with a history of DFSP can be included).
   - Evidence of DFSP expected to be life limiting within the 5 years following the infusion of genetically corrected cells.
10. Known sensitivity to Busulfan.
11. Confirmation of an infectious disease by deoxyribonucleic acid (DNA) PCR positive at time of screening assessment for the following:
    - HIV-1,
    - Hepatitis B,
    - Parvovirus B19.
12. The subject is pregnant or has a major congenital anomaly.
13. Is likely to require treatment during the study with drugs that are not permitted by the study protocol.
14. The subject has previously received another form of gene therapy.

4.3 Subject Withdrawal Criteria

In accordance with the Declaration of Helsinki (and with the applicable country's approval), each subject is free to withdraw from the study at any time for any reason. The Investigator also has the right to withdraw a subject from the study in the event of concurrent illness, AEs, pregnancy (see Section 8.2.4), or other reasons concerning the health or wellbeing of the subject, or in the event of a lack of cooperation. In addition, a subject may be withdrawn from the study as described in Section 3.6.

Should withdrawal occur, all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. A complete final evaluation should be made at the time of the subject's withdrawal (see Section 5.1.8) and an explanation given of why the subject is withdrawing or being withdrawn from the study. The reason for and date of withdrawal from the study must be recorded in the eCRF. If a subject withdraws consent, every attempt will be made to determine the reason. If the reason for withdrawal is an AE or a clinically significant laboratory test abnormality, monitoring will continue until the event has

[2] Dermatofibrosarcoma protuberans is a rare, locally invasive tumor with low metastatic potential that has been reported to be present in some ADA-SCID patients.

resolved or stabilized, or until a cause unrelated to OTL-101 or study procedure has been determined. The specific AE or test result(s) must be recorded in the eCRF. According to the protocol, all evaluations specified for the early termination visit should be carried out.

4.4 Discontinuation/Withdrawal Procedures

If a subject is withdrawn from the study (i.e. ceases participation in the study prior to completion of the assessments planned in the protocol), the primary reason should be recorded in the eCRF. Withdrawal due to AEs should be distinguished from withdrawal due to insufficient response. Where possible the subject should be encouraged to return for the early termination visit assessment to ensure required follow-up is organised for ongoing AEs that may be considered related to OTL-101.

The Investigator will provide or arrange for appropriate follow up (if required) for subjects withdrawing from the study, and will document the course of the subject's condition. Where the subject has withdrawn due to an AE the Investigator should follow the procedures documented in Section 5.1.8 in order to assess the safety of the OTL-101 treatment.

5 STUDY PROCEDURES

The schedule of observations and assessments during the study are summarised in Table 1 and Table 2.

5.1 Study Visits

5.1.1 Screening (Visit 1)

Written informed consent must be obtained prior to any screening procedures (unless routine medical care assessments are being used within 60 days of the harvest visit as screening assessments). A signed and dated informed consent form will be obtained from the parent/legal guardian (according to local law requirements), and a signed and dated assent form will be obtained from each subject (aged ≥7 years), if required according to local regulations. Evaluations obtained as part of routine medical care and performed during the screening period may be used in place of the study specific evaluations if performed within 60 days of the harvest visit (Visit 2: Assessment B), except for cytogenetics, which may be used independently from the time they have been performed. The parents/legal guardians of each subject will acknowledge and agree to the possible use of this information for the study by giving informed consent.

After informed consent is obtained, subjects who are screened will be assigned a 3 digit identification number consecutively.

All screened subjects must be identifiable regardless of eligibility. The Investigator will maintain a list of subject numbers and names with their identification numbers in order to ensure full traceability. Any subject who fails screening may be re-screened at a later date and the same 3 digit identification number will be used.

The study will start with a screening period of up to 60 days prior to harvest (Visit 2: Assessment B).. The screening visit (Visit 1) is defined as the visit during which the informed consent form is signed, before any study-related procedures.

The following assessments will be performed during the screening period so as to determine the subject's eligibility:

- Demographic data: date of birth and sex; ethnicity and/or race according to individual country requirements,
- Complete medical history, including history of ADA-SCID: date of first symptoms, primary diagnosis and date, therapies and responses, stage of disease at diagnosis and at screening.
- Confirmation of ADA-SCID (if not performed before the screening period) based upon biochemical and/or molecular demonstrations of ADA deficiency and T lymphopenia at the time of the initial diagnosis.
- Physical examination, including the recording of height (cm), weight (kg), and vital signs (temperature, pulse rate, respiratory rate and blood pressure). Temperature can be measured as per standard clinic practice.
- Blood tests:
    - Biochemistry (see Table 3),
    - Hematology (see Table 3),

- Prothrombin Time or INR and PTT,
- Peripheral blood or bone marrow for cytogenetic analysis (if cytogenetic testing has not been performed on cells from amniocentesis) by karyotype, Comparative Genome Hybridization (CGH), whole exome sequencing (WES) or other and may be used independently from the time they have been performed,
- HIV-1, HepB and ParvoB19,
- Serum human chorionic gonadotropin (HCG) pregnancy test, if the subject is a female of child-bearing age (to be performed within 30 days of Visit 1),

- Urinalysis,
- Electrocardiogram,
- Echocardiogram,
- Chest X-ray (+/- computer tomography (CT) scan if previous evidence of severe chest disease),
- Pulse oximetry,
- Biopsy of any suspicious skin lesions,
- Evaluation of quality of life, as measured by the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance, and ability to practice sports, respectively,
- Evaluation of severe infections or opportunistic infectious episodes.

In addition, the collection of AEs and concomitant medication will start after signature of the informed consent form.

If a subject meets the eligibility criteria, he/she will be enrolled and scheduled for CD34+ HSCs harvest.

5.1.2 Autologous Bone Marrow Harvest Period (Visit 2)

The source of CD34+ HSCs will be BM. The harvesting of autologous bone marrow will be performed for subjects who meet the inclusion and exclusion criteria at Visit 1. Two assessments will be performed during the hospital admission for BM harvest. This planned hospitalization for protocol procedures will not constitute a SAE.

Pre-harvest procedure (Assessment A)

Once a date for harvest has been scheduled, the pre-harvest procedure will commence up to 5 days beforehand. The results of these pre-operative tests must meet the requirements that permit the harvest of autologous BM, according to standard guidelines in the institution. Pre-harvest tests include:

- Medical history since screening (including AEs and concomitant medications) and physical examination,
- Weight (this measurement will be used for OTL-101 product dose calculation),
- Hematology (see Table 3),
- Prothrombin or INR and PTT,

- Biochemistry; including magnesium and phosphate (see Table 3),
- Type and cross-matching for one unit of packed RBC (PRBC) (to be performed within the period required by the blood bank),
- Serum HCG (pregnancy test) if female of child-bearing age (and if more than 28 days have elapsed since the screening test),
- Urinalysis.

Bone Marrow Harvest (Assessment B)

For BM harvesting, a central venous access device will be required (if not already in place) for ease of phlebotomy and drug administration. The decision between PICC line, a tunneled central venous catheter, or an implanted subcutaneous access device will be made by discussions with subjects parents and line placement surgeon. A PICC line or central venous line may instead be placed as a separate procedure with appropriate sedation or during the general anesthesia for the BM harvest.

The harvest procedure will involve a bilateral BM aspiration in the posterior iliac crests, where a total volume required should be between 15-20 ml/kg of the subjects body weight. The BM sample will be directly transported to the GMP manufacturing location.

Compatible irradiated PRBC (10 to 15 cc/kg) may be transfused following the completion of marrow harvest, if considered appropriate by the Investigator.

If the subject is otherwise well, he/she may be discharged to home, 1-2 days after harvest.

If the first bone marrow harvest fails to collect sufficient HSCs for transplantation or if the product fails to meet the release criteria, the Investigator may perform a second harvest. Screening studies will not be repeated unless 1 year or more has passed from the time of the first harvest.

Any AEs and concomitant medication use should be recorded.

Backup Sample

A back up sample will be taken at the first harvest procedure, in the event of damage during thawing, failure to meet product release specifications or lack of engraftment at 42 days post OTL-101 infusion (see Section 3.4), and must constitute ≥3.0 x 10e7 mononuclear cells/kg.

The backup sample will be cryopreserved, and CD34+ cells will be isolated from the remainder. The backup harvest will consist of non-transduced cells that will be retained if required. A second backup sample is only required if the first backup is not reliable, e.g. if contamination of the initial marrow harvest had occurred.

5.1.3 Baseline (Visit 3)

Pre-Conditioning (Assessment C)

After BM harvest and if the autologous OTL-101 product has met the release criteria (Section 3.7.1), additional baseline samples must be collected for the following tests within 1 week before the first administration of Busulfan:

- Physical examination, including vital signs (temperature, pulse rate, respiratory rate and blood pressure). Temperature can be measured as per standard clinic practice,

- Hematology (see Table 3),
- Prothrombin or INR and PTT,
- Biochemistry (see Table 3),
- Serum HCG (pregnancy test) if female of child-bearing age (and if more than 28 days have elapsed since the screening test),
- Urinalysis,
- Pulse oximetry,
- Interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Measurement of immune function:
    - Absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells,
    - TREC levels,
    - TCR Vβ usage,
    - lymphocyte response to phytohemagglutinin (PHA) (if sufficient cells),
    - Serum immunoglobulin (IgG, IgA, IgM).

  Please note: the above immune function tests can be performed at any point after provision of consent/assent and prior to Busulfan administration in younger subjects if deemed appropriate by the Investigator.
- Peripheral blood mononuclear cells (PBMC) and serum banking for RCL determination. These tests can be performed at any time following BM harvest (Visit 2: Assessment B) and prior to OTL-101 administration (Visit 3: Assessment F) in younger subjects if deemed appropriate by the Investigator.
- Measurements of erythrocyte ADA enzymatic activity and erythrocyte deoxyadenine nucleotide levels. These tests can be performed at any time following BM harvest (Visit 2: Assessment B) and prior to OTL-101 administration (Visit 3: Assessment F) in younger subjects if deemed appropriate by the Investigator.

The Investigator must ensure a PICC line or central venous catheter is in place prior to the start of Busulfan conditioning.

5.1.4 Busulfan Conditioning (Visit 3)

These visits will be performed as part of the subject's hospital admission at Visit 3 (Baseline). Two doses of Busulfan will be administered to the subject as follows:

Day -4 to -3 (Assessment D): The subject's weight will be recorded to calculate the dose of Busulfan. Busulfan will be administered intravenously at a dose of 3 mg/kg infused over 3 hours. Blood samples will be taken immediately at the end of the infusion, and then +1, 2, 4, 8, and 13 hours after the end of infusion, in order to calculate the AUC. These samples may be drawn +/-15 minutes of the time-point, but the actual time with reference to the administration

of Busulfan should be recorded to enable an accurate AUC calculation. Adverse events and concomitant medications will be recorded.

Day -2 to -1 (Assessment E): Busulfan dose adjusted for AUC taken on Day -3. The clinical pharmacist will calculate the AUC from the first dose of Busulfan based on the measured serum levels, to calculate the amount needed for second dose to reach total net AUC of 4,900 (this is equivalent to 20mg*h/L) (see Section 6.2.1.1). Blood samples will be taken before the second infusion (to confirm the previous Busulfan has cleared the blood), immediately at the end of the infusion, and then +1, 2, 4, 8, and 13 hours after the end of infusion, in order to re-calculate the AUC. These samples may be drawn +/-15 minutes of the time-point, but the actual time with reference to the administration of Busulfan should be recorded to enable an accurate AUC calculation. The PK samples will ensure the subject receives the minimum acceptable dose to achieve good engraftment but at the same time minimising the exposure to the conditioning regimen. The tailoring of conditioning treatment is only made possible by using the cryopreserved formulation within this protocol due to the prolonged stability. Adverse events and concomitant medications will be recorded.

Dose Calculation

The calculated AUC from the first Busulfan dose is inserted into the formula below to determine the amount of Busulfan required for the second dose in mg/kg.

(1st dose (mg/kg))/Calculated 1st dose AUC = (Total Dose (mg/kg))/[Target AUC (4,900 uM*min)]

(1st dose (mg/kg))/Calculated 1st dose AUC) x Target AUC (4,900)] = (Total dose (mg/kg))

(1st dose (mg/kg)) x (Target AUC(4,900)]/Calculated 1st dose AUC) = (Total dose (mg/kg))

Calculated total dose (mg/kg) – 1st dose (mg/kg) = 2nd dose (mg/kg).

For example 1st dose = 3 mg/kg and the AUC = 3,200 uM*min

3 mg/kg x 4,900/3,200 = 4.6 mg/kg total dose.

4.6 mg/kg – 3.0 mg/kg = 1.6 mg/kg for second dose.

AUC is calculated from serum Busulfan level values using pKsolver[3].

5.1.5 OTL-101 Administration (Visit 3)

This visit will be performed as part of the subject's hospital admission at Visit 3 (Baseline) and following completion of the conditioning regimen.

Day 0 - day of transplantation (Assessment F)

- Before administration of OTL-101;

3 (https://www.ncbi.nlm.nih.gov/pubmed/20176408) Zhang Y1, Huo M, Zhou J, Xie S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Comput Methods Programs Biomed. 2010 Sep;99(3):306-14. PMID: 20176408

- The subject will be pre-medicated with an appropriate dosage of acetaminophen (e.g., 10 to 15 mg/kg orally), and diphenhydramine (e.g., 0.5 to 1.0 mg/kg intravenous or orally), 15 to 90 minutes prior to infusion.
- Steroids and anticonvulsants will be prepared before infusion and ready to infuse if necessary.
- Serum HCG (pregnancy test) if female of child-bearing age must be performed prior to infusion.

- Monitoring before, during and after the intravenous infusion, as per institution standards, of vital signs and adverse events, including allergic reaction(s). Subjects are required to be closely monitored for signs of allergic reactions/immune reactions, by looking at clinical parameters (fever, rash, difficulties to breath, tremors, tachycardia, arrythmias).
- Administration of OTL-101 product (Day 0) at least 24 hours after the end of the infusion of the last dose of non-myeloablative conditioning (Busulfan). For administration instructions see Section 17: Appendix 1.
- Adverse events and concomitant medications will be recorded.

5.1.6 Immediate Post-transplant Tests and Care (Visit 3)

These visits will be performed as part of the subject's hospital admission which commenced at the baseline visit (Visit 3). During the first month after administration of the OTL-101treatment, the following tests will be performed on the day after treatment (Day 1) and then twice weekly thereafter.

These visits may be performed +/- 2 days from the scheduled date.

Post-transplant (Assessments G to M)

- Interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Hematology (see Table 3),
- Biochemistry (see Table 3).

The subjects will be kept in hospital for close monitoring for up to 24 days post OTL-101 infusion. However, they may be kept in for longer if this is deemed clinically necessary and depending on the medical care they require (e.g. central venous catheter care, infections, protective isolation, nutritional support), the consequences of toxicity due to Busulfan, the engraftment of transduced cells, and immune reconstitution. Alternatively, for subjects who are not geographically close to the clinical site who are clinically stable, post-transplant care may be transferred to the home physician provided the identified clinician agrees to assume this post-transplant monitoring. The home physician will be asked to record all protocol specific assessments on a form provided. This data will be transmitted to the Investigator for data entry into the eCRF. Monitoring can be assured as either an in-patient or out-patient. In the latter case, the subject will stay in hospital for a minimum of 3 days after OTL-101 infusion at the investigational site treatment facility, before being transferred. This planned discharge and subsequent readmission to a second facility would not meet the criteria for being reported as a SAE.

After the transplant, the subject will be maintained on prophylactic antibiotics and intravenous gamma-globulin replacement as needed and/or as per institutional procedures, and will be closely monitored. Any concomitant medication administered to the subject should be recorded in the eCRF. A subject will be readmitted to hospital when deemed clinically necessary. These reasons include, but are not limited to: fever and neutropenia, bacteremia, possible central venous access device infection, or other infections that require initial parenteral antibiotics, organ failure, extreme adverse social situations, and/or bleeding.

5.1.7 Follow-Up (Visits 4 to 11)

Following discharge from the hospital after transplantation, follow-up visits will be performed to determine whether the genetically modified cells have engrafted and are able to produce mature lymphoid and hematopoietic cells containing and expressing the *ADA gene*, and the extent and time-course of immune reconstitution.

The subjects will be evaluated at the following time-points: Month 1, 3, 6, 9, 12, 18, and 24 (end of study visit). Because of the large number of blood tests planned and the limited volumes of blood that can be obtained from young children, it may not be possible to perform every scheduled investigation listed below at each time-point. Therefore, the priority for the ranking of blood tests will be as per the order presented for each visit. The following examinations will be performed:

Month 1/Day 30 (Visit 4 - +/- 1 week))

- Physical examination, vital signs, height, weight and interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Use of PEG-ADA and IgG replacement therapy will be documented. Please note PEG-ADA administration will be stopped at this visit,
- Hematology and biochemistry panels,
- Monitoring for severe infections and/or opportunistic infectious episodes at each study visit,
- Immune function testing;
    - Determination of absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells,
    - Serum immunoglobulin (IgG, IgA, IgM).
- Gene transduction/expression;
    - Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.

Day 42 (Visit 5 - +/- 1 week)

The Day 42 assessment will use laboratory parameters to confirm if hematologic reconstitution has occurred. A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of cell infusion. If hematologic reconstitution has not taken place by Day 42 the backup HSC sample will be processed according to the instructions in Section 3.7

and re-infused to overcome more severe myelosuppressive effects of the conditioning regimen. If the failure of hematologic reconstitution persist after the back-up marrow has been given through day 90 from the initial infusion of the cell product, prolonged unresponsive pancytopenia will exist.

If the Investigator considers the use of the back up HSC sample is not in the best interests of the subject, rescue medication, as per standard institute protocols, will be implemented. Any treatment administered to the subject will be recorded in the eCRF. Adverse events will also be recorded.

Month 3 (Visit 6 - +/- 2 weeks)

- Physical examination, vital signs, height, weight and interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Assessment of re-initiation of PEG-ADA therapy and use of IgG replacement therapy will be documented. Please note: subjects should have discontinued PEG-ADA treatment at Visit 4 (Month 1/Day 30),
- Hematology and biochemistry panels,
- RCL assay in PBMC,
- Serum to be banked for possible Western blot testing for RCL (testing performed if the RCL assay in PBMC returns a positive result),
- Monitoring for severe infections and/or opportunistic infectious episodes at each study visit,
- Vector integration analysis,
- Immune function testing;
    - Determination of absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells:
    - Serum immunoglobulin (IgG, IgA, IgM),
- Gene transduction/expression;
    - Measurement of the proportion of cells containing the inserted *ADA gene* in PBMC and granulocytes and/or lineage-sorted cells,
    - Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.

Month 6 (Visit 7 - +/- 2 weeks)

- Physical examination, vital signs, height, weight and interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Evaluation of quality of life using the Karnofsky/Lansky scale and questions relevant to generall wel-being, school attendance, and ability to practice sports, respectively,
- Assessment of re-initiation of PEG-ADA therapy and use of IgG replacement therapy will be documented. Please note: subjects should have discontinued PEG-ADA treatment at Visit 4 (Month 1/Day 30),
- Assessment of the success of treatment at the subject level ("responder analysis") at 6 months post OTL-101 infusion, by evaluating;

  a) Evidence of erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units),

  b) Evidence of immune reconstitution (absolute number of CD3 cells ≥200/mm3),

  c) Detectable gene-marked granulocytes by differential polymerase chain reaction (dPCR)/qPCR (≥1/10,000 cells).

  If there is no evidence of hematopoietic reconstitution, subjects will require PEG-ADA and/or allogenic HSCT, if available and deemed appropriate by the Investigator. The definition applied for lack of hematopoietic reconstitution is the failure to meet ALL three criteria listed above (a-c). Any subject not meeting any of these three criteria will be deemed a treatment failure and should be withdrawn from the study as described in Section 3.6.1.
- Hematology and biochemistry panels,
- RCL assay in PBMC,
- Serum to be banked for possible Western blot testing for RCL (testing performed if the RCL assay in PBMC returns a positive result),
- Monitoring for leukemia (by nrLAM-PR) if the criteria for triggering nrLAM-PCR are fulfilled. A blood sample will be taken unless clinical/hematologic findings indicate potential leukoproliferation, whereby a bone marrow sample will be required,
- Monitoring for severe infections and/or opportunistic infectious episodes at each study visit,
- Vector integration analysis,
- Immune function testing;
  - Determination of absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells,
  - T-cell proliferative responses to PHA,
  - Measurement of serum immunoglobulin levels (IgG, IgA, IgM) if not receiving immunoglobulins,

- TREC,
- TCR Vβ usage,

- Gene transduction/expression;
  - Measurement of the proportion of cells containing the inserted *ADA gene* in PBMC and granulocytes and/or lineage-sorted cells,
  - Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.

Month 9 (Visit 8 - +/- 2 weeks)

- Physical examination, vital signs, height, weight and interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Assessment of re-initiation of PEG-ADA therapy and use of IgG replacement therapy will be documented. Please note: subjects should have discontinued PEG-ADA treatment at Visit 4 (Month 1/Day 30),
- Hematology and biochemistry panels,
- Monitoring for severe infections and/or opportunistic infectious episodes at each study visit,
- Immune function testing;
  - Determination of absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells;
  - T-cell proliferative responses to PHA,
  - Serum immunoglobulin (IgG, IgA, IgM),
- Gene transduction/expression;
  - Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.

Month 12 (Visit 9 - +/- 4 weeks)

- Physical examination, vital signs, height, weight and interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Evaluation of quality of life using the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance, and ability to practice sports, respectively,
- Assessment of re-initiation of PEG-ADA therapy and use of IgG replacement therapy will be documented. Please note: subjects should have discontinued PEG-ADA treatment at Visit 4 (Month 1/Day 30),
- Hematology and biochemistry panels,
- RCL assay in PBMC,

- Serum to be banked for possible Western blot testing for RCL (testing performed if the RCL assay in PBMC returns a positive result),
- Vector integration analysis,
- Monitoring for leukemia (by nrLAM-PR) if the criteria for triggering nrLAM-PCR are fulfilled. A blood sample will be taken unless clinical/hematologic findings indicate potential leukoproliferation, whereby a bone marrow sample will be required,
- Monitoring for severe infections and/or opportunistic infectious episodes at each study visit,
- Immune function testing;
    - Determination of absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells;
    - T-cell proliferative responses to PHA,
    - Measurement of serum immunoglobulin levels (IgG, IgA, IgM) if not receiving immunoglobulins,
    - Measurement of specific antibodies to tetanus toxoid only if subjects have stopped immunoglobulin replacement and have received tetanus vaccination,
    - TREC,
    - TCR Vβ usage,
- Gene transduction/expression;
    - Measurement of the proportion of cells containing the inserted *ADA gene* in PBMC and granulocytes and/or lineage-sorted cells,
    - Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.

<u>Month 18 (Visit 10 - +/- 4 weeks)</u>

- Physical examination, vital signs, height, weight and interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Evaluation of quality of life using the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance, and ability to practice sports, respectively,
- Assessment of re-initiation of PEG-ADA therapy and use of IgG replacement therapy will be documented. Please note: subjects should have discontinued PEG-ADA treatment at Visit 4 (Month 1/Day 30),
- Hematology and biochemistry panels,
- Monitoring for leukemia (by nrLAM-PR) if the criteria for triggering nrLAM-PCR are fulfilled. A blood sample will be taken unless clinical/hematologic findings indicate potential leukoproliferation, whereby a bone marrow sample will be required,

- Monitoring for severe infections and/or opportunistic infectious episodes at each study visit,
- Vector integration analysis,
- Immune function testing;
  - Determination of absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+(or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells,
  - T-cell proliferative responses to PHA,
  - Serum immunoglobulin (IgG, IgA, IgM),
  - Measurement of specific antibodies to tetanus toxoid only if subjects have stopped immunoglobulin replacement and have received tetanus vaccination.
  - TREC,
  - TCR Vβ usage.
- Gene transduction/expression;
  - Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.

Month 24 (Visit 11 - +/- 4 weeks)

- Physical examination, vital signs, height, weight and interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Evaluation of quality of life using the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance, and ability to practice sports, respectively,
- Assessment of re-initiation of PEG-ADA therapy and use of IgG replacement therapy will be documented. Please note: subjects should have discontinued PEG-ADA treatment at Visit 4 (Month 1/Day 30),
- Hematology and biochemistry panels,
- RCL assay in PBMC,
- Serum to be banked for possible Western blot testing for RCL (testing performed if the RCL assay in PBMC returns a positive result),
- Vector integration analysis,
- Monitoring for leukemia (by nrLAM-PR) if the criteria for triggering nrLAM-PCR are fulfilled. A blood sample will be taken unless clinical/hematologic findings indicate potential leukoproliferation, whereby a bone marrow sample will be required,
- Monitoring for severe infections and/or opportunistic infectious episodes at each study visit,
- Immune function testing;

- Determination of absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+,(memory) T cells
- T-cell proliferative responses to PHA,
- Serum immunoglobulin (IgG, IgA, IgM),
- Measurement of specific antibodies to tetanus toxoid only if subjects have stopped immunoglobulin replacement and have received tetanus vaccination,
- TREC,
- TCR Vβ usage,

- Gene transduction/expression;
  - Measurement of the proportion of cells containing the inserted *ADA gene* in PBMC and granulocytes and/or lineage-sorted cells.
  - Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.

5.1.8 Early Termination Visit

Subjects who meet any of the criteria specified in Section 3.6.1 and Section 4.3 will be encouraged to return for an early termination visit.

Because of the large number of blood tests planned and the limited volumes of blood that can be obtained from young children, it may not be possible to perform every scheduled investigation listed below at each time-point. Therefore, the priority for the ranking of blood tests will be as per the order presented for each visit. The assessments required for the early termination visit are:

- Physical examination, vital signs, height, weight and interval history (including routine review of systems, including infectious symptoms (e.g., fever), AEs and concomitant medications),
- Evaluation of quality of life using the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance, and ability to practice sports, respectively
- Use of PEG-ADA and IgG replacement therapy will be documented,
- Hematology and biochemistry panels,
- RCL assay in PBMC,
- Serum to be banked for possible Western blot testing for RCL (testing performed if the RCL assay in PBMC returns a positive result),
- Monitoring for leukemia (by nrLAM-PR) if the criteria for triggering nrLAM-PCR are fulfilled. A blood sample will be taken unless clinical/hematologic findings indicate potential leukoproliferation, whereby a bone marrow sample will be required.
- Incidence of severe infections and/or opportunistic infectious episodes.
- Immune function testing

- Determination of absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells;
- T-cell proliferative responses to PHA,
- Serum immunoglobulin (IgG, IgA, IgM),
- Measurement of specific antibodies to tetanus toxoid only if subjects have stopped immunoglobulin replacement and have received tetanus vaccination,
- TREC,
- TCR Vβ usage,

- Gene transduction/expression;
  - Measurement of the proportion of cells containing the inserted *ADA gene* in PBMC and granulocytes and/or lineage-sorted cells
  - Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.

Any subject who has received gene therapy treatment with OTL-101 but has subsequently been withdrawn, will be invited to be included in the registry program (see Section 5.1.9).

5.1.9 Post-Study Registry

After completion of the 24 month post-treatment evaluation and end of this study, all subjects receiving OTL-101 will be encouraged to participate in a registry program. Subjects and parents/legal guardians will be asked to provide separate assent/consent to be included in this registry, which will be initiated under a separate protocol.

6 ADMINISTRATION OF OTL-101

6.1 OTL-101

This study does not permit the use of OTL-101 for purposes other than those defined in this protocol. Administration of OTL-101 will be supervised by the Investigator, or designee (as per site personnel list).

All participating subjects will be given the same OTL-101 manufactured from their own harvested autologous $CD34^+$ cells.

6.1.1 Administration of OTL-101

After appropriate thawing (see Section 3.7.3), the OTL-101 productwill be administered via an intravenous infusion over 5 to 15 minutes, according to the instruction described in Section 17: Appendix 1, beginning as soon as possible (and no longer than 30 minutes) after the final thawing steps. The subject will be pre-medicated with an appropriate dosage of acetaminophen (e.g., 10 to 15 mg/kg orally), and diphenhydramine (e.g., 0.5 to 1.0 mg/kg intravenous or orally), 15 to 90 minutes prior to infusion.

Final OTL-101 Dose and Infusion Volume Calculations:

Dose calculation forms are provided in Section 17: Appendix 1. Use the Single Product Bag form unless there is more than one final product bag. Use the Multi-Bag form only if there are two product bags. A form must be completed for each subject and will be used as source data. This form should be filed in the subject's medical notes.

Dose calculation:

1. Prior to Busulfan administration, calculate the total Final Product (FP) CD34+ cells per kg as described on the appropriate worksheet. All values required to perform the calculation will be found on the Certificate of Analysis for the OTL-101 Final Product.
2. Select course of action based on the following criteria:

- The **Total FP $CD34^+$ cells per kg** is less than $2.0x10^6$ $CD34^+$/kg. An additional final product manufacturing process will be required to obtain the minimum acceptable dose,
- The **Total FP $CD34^+$ cells per kg** is greater than $2.0x10^6$ $CD34^+$/kg and less than $20.0x10^6$ $CD34^+$/kg. Thaw and infuse the entire combined final product volume,
- The **Total FP $CD34^+$ cells per kg** is greater than $20.0x10^6$ $CD34^+$/kg. The volume to infuse must be calculated as described on the appropriate worksheet. Do not rinse the partially infused OTL-101 bag after infusion.

The final dose and volume administered to the subjects is required to be recorded in the eCRF.

When more than one bag is needed, only infuse one OTL-101 product bag per hour, and ensure prior to infusion that the weight of the subject is higher than 4 kg to comply with recommended limit of DMSO per kg body weight per day.

6.1.2 Rescue Administration of Autologous HSC Backup

If a subject has not achieved hematopoietic reconstitution at Day 30 (Month 1 visit), he/she will be closely monitored as per standard medical management in order to determine the need for a rescue administration of the autologous back-up. A rescue administration will be given according to the physician's criteria if reconstitution is not observed by Day 42. Subjects may receive transfusions to give more time to observe whether signs of hematopoietic reconstitution are seen. This is not documented in the Schedule of Events tables as rescue procedures would follow standard institute protocols.

- A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of OTL-101 infusion.

If these conditions are fulfilled, the subject's autologous back-up cells will be infused according to standard institute protocols.

If, at Day 90 from the initial infusion of OTL-101, a failure of hematologic reconstitution persists after the subject's autologous back-up cells have been infused and the subject is experiencing prolonged unresponsive pancytopenia, the subject will be withdrawn from the study.

6.2 Concomitant Medication/Therapy

Any prior or concomitant therapy or medication given to a subject from Visit 1 before OTL-101 administration and during the study will be documented in the eCRF. Dose and generic name or tradename will be indicated. The Sponsor will encode all therapy and medication according to the World Health Organization (WHO) drug dictionary.

6.2.1 Permitted Concomitant Medication/Therapy

6.2.1.1 Transplant-related Therapies

Non-myeloablative conditioning with Busulfan

On Day -4 or -3, the subject will be given a loading dose of an anti-convulsant drug (e.g. levetiracetam (10 mg/kg intravenous slow infusion) or clonazepam (0.02 mg/kg orally q 12 hours) prior to the administration of Busulfan, followed by maintenance doses until 24 hours after completion of the Busulfan. Anti-emetic therapy will also be started prior to Busulfan therapy.

Busulfan will be administered in two doses (on Days -4 to -3 and -2 to -1 prior to the day of transplant which is Day 0). The first dose (3 mg/kg intravenous over 3 hours) will be given on Day -4 or -3. Blood samples to determine plasma Busulfan concentrations will be drawn at the following time-points: at the end of the infusion, and then 1, 2, 4, 8, and 13 hours after the end of infusion of the first dose, in order to calculate the AUC. These samples may be drawn +/- 15 minutes of the time-point, but the actual time with reference to the administration of Busulfan should be recorded to enable an accurate AUC calculation.

The AUC will determine the dosage for the second dose, to target a net AUC of 4,900 µM/min (20 mg/l/hr). The pharmacist will calculate the AUC from the first dose based on measured serum levels from the PK.The second dose of Busulfan will be given intravenously on Day -2 or -1 over 3 hours. Busulfan levels will also be measured before the second dose is administered, to ensure the previous dose has cleared the blood, and after the second Busulfan dose to document the concentrations. Blood samples to determine plasma Busulfan concentrations will

be drawn at the following time-points: at the end of the infusion, and then 1, 2, 4, 8, and 13 hours after the end of infusion of the first dose, in order to calculate the AUC. These samples may be drawn +/-15 minutes of the time-point, but the actual time with reference to the administration of Busulfan should be recorded to enable an accurate AUC calculation.

Calculation of the Busulfan dose will be based on weight at admission for transplant. See Section 5.1.4 for the dose calculation instructions.

A "wash-out period" of at least 24 hours will be required between completion of the Busulfan infusion and administration of the OTL-101.

Enzyme replacement therapy with PEG-ADA

1. Immediate ERT after OTL-101 infusion

All subjects included in the study will receive PEG-ADA (Adagen®) prior to OTL-101 infusion, which will be continued until Day 30 (+/-3 days) post-transplant. If, at Day 30 (+/-3 days) post infusion of OTL-101, the subject is not suffering from active infections or other major medical problems, PEG-ADA administration will be discontinued.

2. Rules for re-starting ERT or plan allogenic HSCT (if available)

If, by 6 months after the OTL-101 infusion, there is no evidence of hematopoietic reconstitution following the infusion of the back up OTL-101 and rescue medication/treatment, subjects will require rescue PEG-ADA or allogenic HSCT if available. Positive evidence of hematopoietic reconstitution will require:

a. RBC ADA >0 Units,
b. absolute CD3+ T-cell counts ≥200/mm^3, and
c. peripheral blood samples positive for vector sequences by qPCR (>1/10,000 cells).

In addition, PEG-ADA may be re-started if the Investigator deems it in the best interests of the subject on clinical grounds, e.g. multiple, serious or unresponsive infections or sub-normal immune reconstitution. Once ERT has been re-started, the subject will remain in the study and will continue to be followed-up. If the subject fails to demonstrate hematopoietic reconstitution and has the possibility of receiving an allogeneic transplant, the subject should be withdrawn from the study in order to follow institute transplantation protocols.

Once re-started, PEG-ADA may be discontinued if all the following criteria are met: no active infection, PBMC ADA >6 Units, absolute CD3+ T-cell counts ≥200/mm^3, and peripheral blood samples positive for vector sequences by qPCR.

Prophylaxis for Infections

Prophylactic antimicrobial agents will be used to provide coverage for Pneumocystis jirovecii pneumonia and mucosal yeast:

- Pneumocystis jirovecii prophylaxis (PJP): Trimethoprim/sulfamethoxazole (TMP/SMX), which will be stopped prior to CD34+ cell harvest and then resumed when ANC >500. If, after Day 30, ANC is <500, an alternative PJP prophylaxis regimen will be used. Suitable alternative PJP prophylactic medications include pentamidine (intravenous or aerosolized) or atovaquone. Dapsone should be used with caution,

because of its potential myelosuppressive effects. PJP prophylaxis can be discontinued when CD4 >200/µl (Griffith2009).

- Subjects will continue to receive prophylactic antifungal medication, such as oral nystatin or fluconazole in standard doses. This can be discontinued when CD4 >200/µl.
- Additional or alternative antibiotics may be administered, based on clinical care considerations (these will be recorded as concomitant medications).

Immunoglobulin Replacement Therapy

Immunoglobulin G infusions will be given according to institutional standards (e.g., 400 to 600 mg/kg q 3-4 weeks), and in general are expected to be maintained until through IgG levels of at least 500 mg/dL. Indications for considering the discontinuation of immunoglobulin replacement therapy include: absolute CD4+ >200, absolute B cell >100/µl, IgA or IgM > lower limit of normal for age or gene marking >1% detectable in B cells. IgG levels will be checked monthly to document their independence from IgG infusions. If, at least 3 months later, IgG levels are >300 mg/dL, the subject will undergo vaccination, which will include three doses of tetanus and two to three doses of 13-valent pneumococcal vaccine, according to published clinical standards (Griffith2009). Immunoglobulin G antibodies to tetanus will be determined at least 4 weeks after completing the three vaccinations.

Pre-vaccination and post-vaccination titers will be measured and recorded in the eCRF. Until protective responses to the vaccines have been documented, the subject should remain on antibiotic prophylaxis. If the subject fails to mount a protective antibody response to tetanus, as determined by a titer of anti-tetanus antibodies >0.15 IU/ml at least 4 weeks after the third immunization, IgG replacement therapy will be reintroduced. This therapy will also be resumed in the event of a subject developing an infection that requires hospitalization while off-IgG and on antibiotic prophylaxis.

6.2.2 Prohibited Concomitant Medication/Therapy

It is preferable that subjects should abstain from receiving myelosuppressive medications, including TMP/SMX, during the peri-transplant period. However, if medications that fit this description are clinically warranted, and there are no viable alternatives, then the subject may receive the medication and will be closely monitored.

Subjects should not enrol in any other clinical studies involving gene therapy for 2 years after infusion of the OTL-101 product. Insufficient data are available to predict any interactions with other study agents. Enrolment in other data collection, sample banking, or registry studies is permitted.

6.3 Administration Compliance

The only occasion on which the subjects will be exposed to OTL-101 is the day that they receive their genetically modified cells (Day 0). If a subject fails to demonstrate engraftment/hematopoietic reconstitution at 42 days post initial OTL-101 infusion, their backup sample may be administered. The subjects are required to attend follow-up visits, either at the investigational site or to their personal physician, as per the schedule indicated in Table 1 and Table 2. These follow-up visits are scheduled by the investigational site staff by actively contacting the subject or subject's parents/legal guardians.

7 ASSESSMENT OF EFFICACY

For the timing of efficacy assessments in this study, refer to the schedule in Table 1 and Table 2. Three analyses will be performed during the study, which are described in Section 9.6.

7.1 Efficacy Endpoints

7.1.1 Primary Efficacy Endpoints

The efficacy endpoints for this study include:

1. Evaluation of OTL-101 treatment at 6 months post administration by assessing, in support of CMC assessing comparability of cryopreserved and fresh formulations, evaluation of therapy success for each subject based on the following parameters and their thresholds:

   d) Erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units),

   e) Absolute CD3+ T-cell counts ≥ 200/mm3, and

   f) Peripheral blood samples positive for vector sequences by qPCR(≥1/10,000 cells).

   Please note: subjects must meet all three criteria. Subjects not meeting any of these criteria will be designated a failure (non-responder) and will be withdrawn from the study (Section 3.6.1).

   The proportion of responders in this study will be compared with the proportion of responders in one of the Phase I/II ongoing studies using the fresh formulation.

2. Evaluate the overall survival and event free survival 12 months post OTL-101 infusion. Overall survival is defined as the proportion of subjects alive. Event-free survival is defined as the proportion of subjects alive with no “event”; an “event” being the resumption of PEG-ADA ERT or the need for a rescue allogenic HSCT, or death.

The efficacy assessments are described in Section 7.2.

7.1.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints include:

- Overall survival and event free survival 24 months post OTL-101 infusion.
- Use of immunoglobulin replacement therapies prior to and after gene therapy.
- Performance outcomes and quality of life measured by the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance and ability to practice sports, respectively.
- Rate of severe infections/opportunistic infectious episodes, defined as infections requiring hospitalization or prolonging hospitalization and/or documented infections by opportunistic pathogens (e.g. interstitial pneumonia, intractable diarrhoea).
- Response to tetanus vaccination.
- Immune reconstitution: T and B cell reconstitution.

The efficacy assessments are described in Section 7.2.

7.1.3 Exploratory Efficacy Endpoints

The following laboratory correlates of efficacy will be used to assess the level of gene correction, engraftment and immune reconstitution:

- Quantification of clonal diversity of vector integrants.
- TREC and FACS for TCR V-beta family use.
- Percentage gene marking in peripheral blood cells.
- Adenosine deaminase enzyme activity in erythrocytes.
- Total adenine nucleotides in erythrocytes.
- Vector integration analysis.
- Immune reconstitution
    - Absolute Lymphocyte Count.
    - Absolute numbers of T, B, NK lymphocytes in peripheral blood.
    - T lymphocyte proliferative responses to mitogen (PHA) and to antigens (tetanus toxoid after vaccination)
    - Serum immunoglobulin levels (IgG, IgA and IgM).

The efficacy assessments are described in Section 7.2.

7.2 Efficacy Assessments

The timing of assessing efficacy data are discussed in Section 5 and summarised in Table 1: Schedule of Events – Screening to Day 24 and Table 2: Schedule of Events – Month 1 to Month 24.

Immune Function

Immune function tests will include the phenotyping of lymphocyte subsets, the quantification of immunoglobulins ((IgG, IgA, IgM) and antibodies to Tetanus toxoid, and T-cell proliferation to PHA.

Ongoing T lymphopoiesis will be assessed by quantifying TREC in blood cells at different time points after transplant using qPCR with genomic DNA extracted from PBMC or CD3-enriched cells (Clinical Immunology Laboratory at UCLA).

Immune function testing will be assessed using the following endpoint assays:

- Absolute lymphocyte counts will be determined by routine clinical laboratory tests.
- Absolute numbers of T, B, NK lymphocytes in peripheral blood will be determined by flow cytometry.
- T lymphocyte proliferative responses to mitogen (PHA) will be determined by tritiated thymidine incorporation or Carboxyfluorescein succinimidyl ester (CFSE) analysis by routine clinical laboratory tests.
- TREC and FACS for TCR V-beta family use will be analyzed by measuring PBMC or CD3 TREC by qPCR, and TCR Vβ family use by FACS and deep sequencing.

- Serum immunoglobulin levels (IgG, IgA and IgM) will be determined using routine clinical laboratory tests.
- Serum titers of isohemagglutinin antibodies and antibodies to vaccine antigen tetanus toxoid will be determined by routine clinical laboratory tests.

Tests Related to the Efficacy of Gene Transfer

The following will be measured:

- Measurements of erythrocyte ADA enzymatic activity and deoxyadenine nucleotides in RBC (RBC dAXP, RBC ADA activity),
- Quantitative PCR for vector sequences will be used to measure the frequency of cells containing the inserted *ADA gene* in PBMC and granulocyte fractions, as well as FACS-sorted T-cells, B-cells, NK cells, and myeloid cells when sufficient samples are available.
- Serum and PBMC samples will be used to detect RCL.

Gene Transduction/Expression

Percentage Gene Marking in PB cells

The efficacy of stem cell transduction/engraftment will be determined by serial quantitative DNA-PCR examinations of PBMC and granulocytes in order to quantify the percentages of cells containing ADA cDNA. Serial samples of peripheral blood will be collected and fractionated to obtain leukocytes on Ficoll-Hypaque to obtain PBMC and granulocyte populations. Genomic DNA isolated from each cell population will be assayed for the frequency of cells containing the EFS-ADA LV , using qPCR. If sufficient cells are available, then the PBMC cells may be sub-fractionated by immunoaffinity or FACS sorting into T-cells (CD3+), B-cells (CD19+), NK cells (CD16+/CD56+) and myeloid cells (CD13+/CD14+, or CD33+), and DNA from these cell sub-populations will be assayed by qPCR for vector marking. The presence of transduced peripheral blood leukocytes will demonstrate transduction and engraftment of the genetically manipulated CD34+ cells. An absence of transduced cells will indicate a failure of transduction and/or engraftment.

Clonal Diversity of Vector Integrants

Clonal diversity will be quantified and used as an index for the numbers of vector transduced HSC that have engrafted in the subjects. The number of LV integration sites will be amplified using a modified nrLAM-PCR protocol (Paruzynski 2010) and sequenced via Illumina GAIIx high-throughput sequencers. A custom-made software code has been written to process raw sequence reads and determine the genomic positions of integration sites. We expect to identify >1 x 10e5 integration sites per experiment, which will lend significant statistical power to downstream analysis.

Adenosine deaminase Enzyme Activity in Erythrocytes

Adenosine deaminase enzymatic activity in erythrocytes will be measured for all subjects at the Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory at Duke University under the direction of Michael Hershfield. In this laboratory, the reference range for ADA enzyme activity in erythrocytes from normal donors is defined. Actual levels of ADA enzyme activity, and the attainment of sufficient enzyme levels within the normal reference

range, and similar to those of the normal population with functional immune systems, will be recorded.

Total Adenine Nucleotides in Erythrocytes

The levels of deoxyadenine nucleotides in erythrocytes will be determined at each time point (at the laboratory headed by Dr. Michael Hershfield, Duke University). The level of deoxyadenine nucleotides in erythrocytes provides an indirect assessment of systemic detoxification by ADA enzyme activity. While not a primary study endpoint, this parameter will be measured to provide additional information on the effects of the procedure.

Quality of Life

An assessment will be made of general quality of life and neurological development according to the schedule of events tables. During these visits, the impact of illness on health-related quality of life will be determined using the Karnofsky/Lansky performance scale (which is recommended for immune deficiencies and transplanted patients) (see Section 17: Appendix 2).

Questions relevant to general well-being, school attendance and ability to practice sports, will also be posed to the subject and his/her parents/legal guardians by the Investigator. These questions will form part of the eCRF. Questions suitable/relevant to the age of the subject will be posed.

Infections

Although infections are safety evaluations, in ADA-SCID, they are also a clear feature of the disease. Evaluation of the frequency of severe infections or opportunistic infectious episodes is an important measure to evaluate efficacy (immune-reconstitution). This is defined as infections or severe infections requiring hospitalization or prolonging hospitalization and/or documented infections by opportunistic pathogens (e.g. interstitial pneumonia, intractable diarrhoea).

8 ASSESSMENT OF SAFETY

For the timing of safety assessments in this study, refer to the schedule of events in Table 1 and Table 2.

The principal Investigator is responsible for the safety of subjects included in the study. The safety of this study will be overseen by an overarching and independent Data Safety Monitoring Committee Board.

8.1 Safety Endpoints

The safety and tolerability of OTL-101 will be assessed throughout the study by evaluating AEs, clinical laboratory test results, vital signs measurements, ECG, physical examination results, and concomitant medication usage. This will include the occurrence of events related to safety:

- Grade 3/4 procedure-related AEs, including GvHD.
- Episodes of severe and opportunistic infections.
- Clinical laboratory toxicities, including myelosuppression, abnormal blood cell counts and/or biochemistry parameters, according to AE grading scale described in Section 8.2.1.4.
- Toxicity related to the use of LV, specifically the emergence of replication competent lentivirus or monoclonal expansion or leukemia, due to vector insertion.

The safety assessments are described in Section 8.2.

8.2 Safety Assessments

8.2.1 Adverse Events

Adverse events will be monitored and recorded from the time that the subject's parents/legal guardians provide informed consent and then throughout the study (see Section 3.5 for definition of the study duration) and will be elicited by direct, non-leading questioning or if spontaneously reported by the subject/parent/legal guardian. Further details on AE reporting can be found in Section 8.2.2.

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not it is considered to be causally related to the product. An undesirable medical condition can be symptoms (e.g. nausea, chest pain), signs (e.g. tachycardia, enlarged liver) or the abnormal results of an investigation (e.g. laboratory findings, ECG). In clinical studies an AE can include an undesirable medical condition occurring at any time, including run in or washout periods, even if no OTL-101 has been administered.

Death due to disease progression will be recorded as part of the efficacy evaluation (due to failure of the OTL-101 product) and will be regarded as an SAE unrelated to OTL-101.

8.2.1.1 Severity Classification of Adverse Events

Adverse events will be recorded and graded according to an adapted Pediatric Clinical Toxicity Scale from the National Institute Allergy and Infectious Diseases (NIAID), Autoimmuno-deficiency Syndrome (AIDS) Division (see Section 17: Appendix 3):

Grade 1 (Mild): Symptoms causing no or minimal interference with usual social & functional activities.

Grade 2 (Moderate): Symptoms causing greater than minimal interference with usual social & functional activities.

Grade 3 (Severe): Symptoms causing an inability to perform usual social & functional activities.

Grade 4 (Life threatening): Symptoms causing an inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death.

Grade 5 (Death)

8.2.1.2 Causality Classification

The relationship between an AE and study product administration will be classified according to the following:

Related: reports that contain good reasons and sufficient information (e.g. plausible time sequence, dose response relationship, pharmacology, positive de-challenge and/or re-challenge) to assume a causal relationship with OTL-101 administration in the sense that it is plausible, conceivable or likely.

Not related: reports that contain good reasons and sufficient information (e.g. implausible time sequence and/or attributable to concurrent disease or other drugs) to rule out a causal relationship with OTL-101 administration.

8.2.1.3 Assessment of Expectedness

The reference document used to assess the expectedness of AEs in this study will be the current IB.

8.2.1.4 Laboratory Test Abnormalities

Abnormalities in laboratory test values should only be reported as AEs if any of the following apply:

- They result in a delay of the OTL-101 treatment or other study procedures,
- They require an intervention or diagnostic evaluation to assess the risk to the subject,
- They are considered to be clinically significant by the Investigator.

8.2.1.5 Abnormal Physical Examination Findings

In the judgment of the Investigator, clinically significant changes to the findings of physical examinations (abnormalities) will be recorded as AEs.

8.2.1.6 Other Investigation Abnormal Findings

Abnormal test findings judged by the Investigator to be clinically significant (e.g. ECG, echo changes) and that require an intervention or diagnostic evaluation to assess the risk to the subject, should be recorded as AEs.

8.2.2 Recording and Follow up of Adverse Events

At each study visit, the subject (or parent/legal guardian) should be asked a non-leading question such as: “How have you felt since transplantation (or last visit)?” All observed or voluntarily reported AEs, regardless of their potential causal relationship with OTL-101, will be recorded on the AE page(s) of the eCRF. Events involving drug reactions, accidents,

illnesses with onset during the study, or exacerbations of pre-existing illnesses should be recorded. Any AEs already recorded and designated as 'continuing' should be reviewed at each subsequent assessment.

Any AEs reported at visits to the subject's general practitioner must be transmitted by the healthcare professional to the responsible investigational site staff, if possible on the same day, so that they can be recorded in the eCRF, notified to the Sponsor (if an SAE), and followed up appropriately by the responsible investigational site staff.

For all AEs, the Investigator must pursue and obtain information that is adequate both to determine the outcome of the AE and to assess whether it meets the criteria for classification as an SAE requiring immediate notification to the Sponsor or its designated representative. For all AEs, sufficient information should be obtained by the Investigator to make an assessment of the causality of the AE (i.e. protocol treatment or other illness). The Investigator is required to assess causality and record that assessment on the eCRF. Follow up of the AE, after the date of OTL-101 infusion, is required if the AE or its sequelae persist. Follow up is required until the event or its sequelae resolve or stabilize at a value acceptable to the Investigator and the Sponsor's clinical monitor or his/her designated representative.

8.2.3 Reporting of Serious Adverse Events

All SAEs (as defined below) regardless of the suspected relationship to OTL-101, must be reported immediately (within 24 hours of the Investigator's knowledge of the event) to the pharmacovigilance contact specified at the beginning of this protocol. If the immediate report is submitted by telephone, this must be followed by detailed written reports using the SAE report form.

For subjects who are not geographically close to the clinical site who are clinically stable, post-transplant care may be transferred to the home physician provided the identified clinician agrees to assume this post-transplant monitoring. Any discharge from the clinical site and re-admission to the local site (second facility) would not meet the criteria for being reported as a serious adverse event.

An SAE is any AE that:

- Results in death,
- Is life threatening, that is any event that places the subject at immediate risk of death from the event as it occurred. It does not include an event that, had it occurred in a more severe form, might have caused death,
- Results of in-patient hospitalization or a prolongation of existing hospitalization, excluding admission for social or administrative reasons (see below),
- Results in a persistent or significant disability/incapacity, where disability is a substantial disruption of a person's ability to conduct normal life functions,
- Results in congenital anomaly/birth defect in the offspring of a subject who received OTL-101,
- Is an important medical event that may not result in death, be life threatening, or require hospitalization, but that, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm

requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

In addition to the above criteria, any additional AE that the Sponsor or an Investigator considers serious should be reported immediately to the Sponsor and included in the corporate SAE database system.

- Hospitalization is defined as any in-patient admission (even if less than 24 hours). For chronic or long term in-patients, in-patient admission also includes transfer within the hospital to an acute/intensive care in-patient unit.
- Prolongation of hospitalization is defined as any extension of an in-patient hospitalization beyond the stay anticipated/required in relation to the original reason for the initial admission, as determined by the Investigator or treating physician. For protocol specified hospitalization in clinical studies, prolongation is defined as any extension beyond the length of stay described in the protocol. Prolongation in the absence of a precipitating, treatment emergent, clinical AE (i.e. not associated with the development of a new AE or worsening of a pre-existing condition) may meet criteria for "seriousness" but is not an adverse experience and thus is not subject to immediate reporting to the Sponsor.
- Preplanned or elective treatments/surgical procedures should be noted in the subject's screening documentation. Hospitalization for a preplanned or elective treatment/surgical procedure should not be reported as an SAE unless there are complications or sequelae which meet the criteria for seriousness as described above.

Any SAE must be reported immediately (within 24 hours), independent of the circumstances or suspected cause, if it occurs or comes to the attention of the Investigator at any time during the study period.

Any AE/SAE with a suspected causal relationship to OTL-101 administration occurring at any other time after completion of the study must be promptly reported. The following information is the minimum that must be provided to Sponsor's pharmacovigilance contact within 24 hours for each SAE:

- Study number
- Center number
- Subject number
- AE
- Investigator's name and contact details

The additional information included in the SAE form must be provided to the Sponsor or nominated representative as soon as it is available. The Investigator should always provide an assessment of causality for each event reported to the Sponsor. Upon receipt of the initial report, the Sponsor will ask for the Investigator's causality assessment if it was not provided with the initial report.

The Investigator should report a diagnosis or a syndrome rather than individual signs or symptoms. The Investigator should also try to separate a primary AE considered as the foremost untoward medical occurrence from secondary AEs which occurred as complications.

8.2.4 Pregnancy

Pregnancy itself is not regarded as an AE unless there is a suspicion that OTL-101 has interfered with a contraceptive method. If pregnancy occurs during the study, the outcome of the pregnancy will then need to be collected (post-study if necessary).

The subjects recruited into this study are under the age of 18 years, however it is anticipated that most subjects will be pre-pubescent. A serum HCG pregnancy test will be performed on all female subjects of childbearing potential at screening, baseline/pre-conditioning assessment, before the administration of Busulfan, and before the administration of OTL-101. For females of child-bearing potential in whom preventive measures fail, pregnancies will be reported as indicated below.

In the event of any pregnancy reported in a participant no protocol treatment will be administered. Any subject who becomes pregnant during the study, before the administration of Busulfan or before the administration of OTL-101, will be withdrawn.

Information regarding pregnancies must be collected on the AE page of the eCRF and reported to the Sponsor as an SAE. The Sponsor will request further information from the Investigator as to the course and outcome of the pregnancy.

The Investigator must instruct all female subjects of reproductive age to use a medically approved method of contraception throughout study participation. The Investigator must also instruct all female/male subjects of reproductive age to inform them immediately should they/or their partner become pregnant during the study. The Investigator should counsel the subject, discuss the risks of a pregnancy and the possible effects on the fetus. Monitoring of the subject/the partner should continue until conclusion of the pregnancy, which may involve follow up after the subject's involvement in the study has ended.

If the Investigator becomes aware of a pregnancy occurring in the partner of a subject participating in the study, this should be reported to the Sponsor. After the partner has given written consent, she should be counselled and followed as described above. Monitoring of the partner should continue until conclusion of the pregnancy.

8.2.5 Deaths

All AEs resulting in death during the study period must be reported as an SAE within 24 hours of the Investigator's knowledge of the event.

The convention for recording death is as follows:

- Adverse event term: lead cause of death (e.g. multiple organ failure, pneumonia, myocardial infarction),
- Outcome: fatal.

The **only exception** is if the cause of death is unknown (i.e. sudden or unexplained death), in which case the AE term may be 'Death' or 'Sudden death'.

8.2.6 Discontinuation/Withdrawal due to Adverse Events/Serious Adverse Events

Discontinuation/withdrawal due to AEs should be distinguished from discontinuation/ withdrawal due to an insufficient response to OTL-101.

If the subject withdraws from the study due to an AE/SAE, it must be reported immediately to the Sponsor's designated representative (see Section 8.2.3). In all cases, the Investigator must ensure the subject receives appropriate medical follow up.

8.2.7 Reporting to Competent Authorities/IECs/IRBs/Other Investigators

The Sponsor will ensure that processes are in place for the submission of reports of Suspected Unexpected Serious Adverse Reactions (SUSARs) occurring during the study to the CA, IEC/IRB and other Investigators concerned by OTL-101. Reporting will be done in accordance with the applicable regulatory requirements.

For the single study center in the US, SUSARs and other important safety reports, when appropriate, will be submitted directly to the Investigators. It is the responsibility of Investigators to notify their IEC/IRB and the FDA in a timely manner.

8.3 Physical Examination

Physical examinations, including weight (kg) and body height (cm) will be conducted at times indicated in Table 1 and Table 2.

Any clinically significant physical examination findings (abnormalities) observed during the study will be reported as AEs. Any physical examination findings (abnormalities) persisting at the end of the study will be followed by the Investigator until resolution or until reaching a clinically stable endpoint.

A review of all body systems will include the following:

- General appearance
- Skin
- Head, including ears, eyes, nose, throat, if feasible.
- Respiratory
- Cardiovascular
- Abdomen (including liver and kidneys)
- Neurological examination with sensory testing

Any abnormalities or changes in intensity noted during this review of body systems should be documented in the source document and reported appropriately in the eCRF. In addition, the resolution of any abnormal findings during the study will be noted in the source document and eCRF, if clinically significant.

8.4 Vital Signs

Vital Signs (including blood pressure, pulse rate, respiratory rate and temperature) will be conducted at times indicated in Table 1 and Table 2. Blood pressure and pulse will be recorded after five minutes of rest in a sitting position.

Any clinically significant values or deviations from baseline vital signs will be recorded as an AE.

8.5 Electrocardiograms

An ECG will be conducted at times indicated in Table 1 and Table 2.

A twelve-lead ECG will be recorded at a paper speed of 25 mm/sec so that the different ECG intervals (RR, PR, QRS, QT) can be measured manually/automatically. The ECG will be recorded [with the subject in supine position after five minutes of rest] until four regular consecutive complexes are available. Automated ECG interval estimates taken from the ECG recorder will be used in this study.

Any clinically significant abnormalities will be recorded as AEs.

8.6 Laboratory Safety Tests

Blood samples for serum chemistry and hematology, and a urine sample for urinalysis will be taken for evaluation of laboratory safety parameters listed in Table 3 according to the times indicated in Table 1 and Table 2. All clinical laboratory assays will be performed according to the relevant laboratory's normal procedures. Reference ranges will be supplied by the laboratory and used to assess the laboratory data for clinical significance and out-of-range pathological changes.

For the evaluation of hematology and biochemistry panels, and urinalysis, the Investigator will review the safety laboratory test results, document the review, and record any clinically relevant changes occurring or observed during the study in the AE section of the eCRF. Changes compared to pre-harvest tests will be recorded as AEs if they are considered to be clinically significant by the Investigator.

All clinically relevant abnormal laboratory tests occurring during the study will be repeated at appropriate intervals until they return to baseline or to a value deemed acceptable by the Investigator and the Sponsor's clinical monitor (or his/her designated representative) or until the abnormality is explained by an appropriate diagnosis.

Table 3: Clinical Laboratory Tests

Hematology parameters	**Biochemistry parameters**	**Urinalysis**
Red blood cell (RBC) count	Blood Urea Nitrogen	pH
Hemoglobin	Creatinine	Glucose
Hematocrit	Total bilirubin	Ketones
Mean corpuscular volume (MCV)	Sodium	Protein
Mean corpuscular hemoglobin (MCH)	Potassium	Bilirubin
Mean corpuscular hemoglobin concentration (MCHC)	Chloride	Blood
White blood cell (WBC) count with differential;	Calcium	Urobilinogen
Neutrophils	Aspartate transaminase (AST)	Nitrites
Lymphocytes	Alanine transaminase (ALT)	Leukocytes
Monocytes	Alkaline phosphatase (ALP)	Specific gravity
Eosinophils	Albumin	
Basophils	Total protein	
Platelet count	Glucose	
Coagulation parameters	Magnesium	
Prothrombin time (PT) or partial thromboplastin time (PTT) and internal normalised ratio (INR)	Phosphates	

8.6.1 Biochemistry

Blood samples for biochemistry will include the assessment of parameters listed in Table 3, according to the times indicated in Table 1 and Table 2.

Any unscheduled test (indication and results) will be recorded on the dedicated page in the eCRF.

8.6.2 Hematology

Blood samples for hematology will include the assessment of parameters listed in Table 3, according to the times indicated in Table 1 and Table 2.

Any unscheduled test (indication and results) will be recorded on the dedicated page in the eCRF.

8.6.3 Urinalysis

Fresh urine samples will be collected and will include the assessment of parameters listed in Table 3, according to the times indicated in Table 1 and Table 2. Any abnormalities detected on dipstick at Screening (Visit 1), Pre-harvest (Visit 2, Assessment A) and Baseline (Visit 3, Assessment C) will require repeat dipstick and microscopic examinations.

Microscopic tests will be performed, if indicated, but results will not be collected in the eCRF. If, in the opinion of the Investigator, there are any clinically significant abnormalities under microscopy, they will be recorded as an AE in the eCRF.

8.6.4 Pregnancy Test

Serum HCG pregnancy tests will be performed on all females of child-bearing potential at times indicated in Table 1.

8.6.5 HIV-1, HepB, ParvoB19

These tests will be performed at baseline to determine eligibility.

8.7 Blood Volume

The total volume of blood drawn for each assessment visit is estimated to be between 15 to 90 ml (2 to 6 tablespoons) for each subject.

8.8 Pharmacokinetic Evaluations of Busulfan

Blood samples to determine plasma Busulfan concentrations will be drawn after the first and second doses of Busulfan to calculate the AUC at times indicated in Table 1.

9 STATISTICAL METHODS

9.1 Background

The primary objective of this clinical study is to further explore the safety and efficacy of OTL-101, an autologous, genetically modified CD34+ HSC based gene therapy for the treatment of ADA-SCID. The study will also be supportive of the analytical CMC comparability studies between fresh and cryopreserved OTL-101 formulations. The clinical outcomes (good predictors of survival and event free survival): RBC ADA >0 Units, absolute CD3+ T-cell counts $\geq 200/mm^3$, and peripheral blood samples positive for vector sequences by qPCR(>1/10,000 cells) will be assessed as part of an interim analysis following completion of the 6 month follow-up visit.

9.2 Design Considerations and Data

This is a prospective, non-randomized, single-cohort, longitudinal, single-center, clinical study aimed to further evaluate the safety and efficacy of OTL-101.

Descriptive results will be presented for the study cohort.

9.3 Study Endpoints

The efficacy, including exploratory endpoints, are detailed in Section 7.1 and the safety endpoints are detailed in Section 8.1.

9.3.1 Primary Efficacy Endpoints

The primary efficacy endpoints for this study include treatment success at 6 months post OTL-101 infusion, and overall survival and event-free survival at 12 months post OTL-101 infusion.

Treatment success at 6 months post OTL-101 infusion, will be performed in support of CMC assessing comparability between cryopreserved and fresh formulations. Evaluation of the success of therapy will be based on the following parameters and their thresholds:

a) Erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units),

b) Absolute CD3+ T-cell counts $\geq 200/mm^3$, and

c) Peripheral blood samples positive for vector sequences by qPCR(≥1/10,000 cells).

Treatment will be deemed sucessful in subjects meeting all three above thresholds. These subjects will be designated as “responders”. Those subjects failing to meet at least one of the above thresholds will be considered to be “non-responders.”

Overall Survival is defined as the time-to death from the OTL-101 infusion if death occurred, or to the date of the last evaluation if death did not occur. If no death occurred, the subject’s data will be considered censored at the last evaluation.

Event Free Survival is defined as the time-to event from the OTL-101 infusion if an event occurred, or to the date of the last evaluation without an event if no event occurred. If no event occurred, the subject’s data will be considered censored at the last evaluation. Event is defined as any of the following:

- Death
- Returning to PEG-ADA ERT
- Need of rescue HSCT

9.3.2 Secondary Efficacy

The secondary efficacy endpoints will be assessed at 12 and 24 months post OTL-101 administration and include:

- Overall survival and event free survival 24 months post OTL-101 infusion,
- Use of immunoglobulin replacement therapies prior to and after OTL-101 infusion,
- Assessment of performance outcomes and quality of life measured by the Karnofsky/Lansky scale and questions relevant to general well-being, school attendance and ability to practice sports, respectively,
- Rate of severe infections/opportunistic infectious episodes, defined as infections requiring hospitalization or prolonging hospitalization and/or documented infections by opportunistic pathogens (e.g. interstitial pneumonia, intractable diarrhoea),
- Response to tetanus vaccination,
- Immune reconstitution: T and B cell reconstitution.

All summaries of secondary endpoints will be descriptive.

9.3.3 Exploratory Efficacy Endpoints

The following laboratory correlates of efficacy will be used to assess the level of gene correction, engraftment and immune reconstitution:

- Quantification of clonal diversity of vector integrants.
- TREC and FACS for TCR V-beta family use.
- Percentage gene marking in peripheral blood cells.
- Adenosine deaminase enzyme activity in erythrocytes.
- Total adenine nucleotides in erythrocytes.
- Vector integration analysis.
- Immune reconstitution
    - Absolute Lymphocyte Count.
    - Absolute numbers of T, B, NK lymphocytes in peripheral blood.
    - T lymphocyte proliferative responses to mitogen (PHA) and to antigens (tetanus toxoid after vaccination)
    - Serum immunoglobulin levels (IgG, IgA and IgM).

All summaries of exploratory endpoints will be descriptive.

9.3.4 Safety Endpoints

Safety endpoints in this study are AEs, including SAEs, with severity graded by Common terminology criteria for adverse events (CTCAE) criteria, including relation to treatment. Special attention will be paid to the following AE types:

1. Clinical toxicities
2. Exposure to RCL

3. Development of monoclonal expansion or leukoproliferative complications from vector insertional effects.

9.4 Sample Size Considerations

Due to the ultra-orphan nature of this indication, the sample size for this study is based on practical considerations. Specifically, the number of ADA-SCID patients expected to meet eligibility criteria yearly is around four to five. With a planned enrollment of three to six subjects per year, the sample size (N=10) for this study is considered reasonable.

It should be noted that the sample size will provide precision of 15% for 100% success (survival) at any particular time point. Precision is here defined as the half-width of the two-sided 95% CI using the Exact Binomial computations.

Ten subjects receiving OTL-101 will participate in this study.

Although all subjects will be evaluable for the first analysis at 6 months post OTL-101 infusion, the first five subjects will be reviewed to monitor the outcome of the study treatment. Sample size considerations for the first analysis of five subjects completing the 6-month follow-up assessment is based on realistic enrollment for this orphan indication. Experts in the field of HSCT and gene therapy use RBC ADA enzyme activity >0 U, absolute CD3+ T-cell counts $\geq 200/mm^3$, and peripheral blood samples positive for vector sequences by qPCR (>1/10,000 cells) as predictors of survival and event free survival and ultimately the success of the OTL-101 treatment. These parameters achieve plateau between 3 and 4 months post-intervention, therefore, a 6-month data cut for this interim analysis should be indicative of the final study outcomes.

9.5 Analysis Populations

9.5.1 Efficacy Population

The efficacy population will be a modified intent-to-treat population including all subjects treated with OTL-101 within this protocol.

9.5.2 Safety Population

The safety population will consist of all subjects treated with OTL-101 within this protocol.

9.5.3 Pharmacokinetic Population

The PK population will include all subjects who have received at least one dose of Busulfan and for whom there are sufficient plasma samples to allow reliable determination of PK profile. Subjects' evaluability will be determined by the Investigator.

9.6 Statistical Methods

9.6.1 Overview

Full statistical methods will be detailed in the statistical analysis plan (SAP). The data will be summarized in tables displaying the mean, standard deviation, median, minimum, maximum and number of subjects for continuous data (e.g. age, weight) or in tables displaying count and percentage for categorical data (e.g. gender, previous treatment). Data will be presented by visit, if applicable.

All statistical analyses will be performed using Statistical Analysis System (SAS)® Version 9.4. or higher.

Statistical analyses will be performed, and their outcomes presented, in three stages:

1. The first (interim) analysis will compare the success/failure data 6-months post OTL-101 infusion. The success/failure of the OTL-101 cryopreserved formulation will be defined by the three criteria listed in the primary objectives (Section 2.2.1). This data will be compared with available data from the ongoing UCLA Phase I/II study using the OTL-101 fresh formulation. This will support the CMC comparability data between OTL-101 cryopreserved and fresh formulations. Secondary and exploratory endpoints will also be described.
2. The second (primary) analysis will determine the 12 month overall survival and event free survival for all subjects. Secondary and exploratory endpoints, as well as baseline characteristics, subject disposition, and safety data will also be described.
3. The third (final) analysis will be performed to determine the overall survival and event free survival for all subjects 24 months post OTL-101 infusion. Secondary and exploratory endpoints, as well as baseline characteristics, subject disposition, safety and Busulfan PK data will also be described.

This study is not designed to be powered to demonstrate statistical significance; therefore, no correction for overall Type I Error will be performed.

9.6.2 Subject Disposition

Subject disposition will be tabulated; the number of enrolled, exposed, prematurely terminated, ongoing and completed subjects will be summarized. A list of dropouts will be prepared including treatment received, reason for discontinuation, and time of discontinuation.

9.6.3 Baseline Characteristics

The following information collected at baseline will be presented:

- Demographics (Age, Gender, etc.),
- Method of diagnosis,
- Previous HSCT including: date, transplant cells, donor type and outcome,
- Previous PEG-ADA ERT (and duration of treatment),
- Previous or current immunoglobulin therapy,
- Listing of relevant medical history, including infections.

9.6.4 Efficacy Analyses

The primary efficacy endpoints will be summarized as follows:

Responder Analysis

The proportion of responders in this study will be summarized and compared with the proportion of responders in the Phase I/II ongoing studies using the fresh formulation.

Overall and Event Free Survival

Overall and event free survival will be summarized using:

- Proportion of patients who survived at each specified analysis timepoint, with the exact binomial 95% confidence interval.
- Kaplan-Meier survival curve of time to death/event.

All efficacy summaries will be descriptive, there will be no formal statistical comparisons.

9.6.5 Safety Analyses

All AEs will be coded according to the current Medical Dictionary for Regulatory Activities (MedDRA) version and will be classified by MedDRA preferred term and system organ class. AE listings will be presented by subject, system organ class and preferred term. Incidence of treatment emergent AEs (TEAE) and SAEs will be tabulated by system organ class and preferred term. In addition, summary tables will be presented by maximum severity, relationship to treatment and TEAEs associated with premature withdrawal from the study.

A TEAE is defined as any AE that occurs during the active phase of the study if:

- it was not present prior to receiving the OTL-101 infusion, or
- it was present prior to receiving the OTL-101 infusion but the intensity increased during the active phase of the study, or
- it was present prior to receiving theOTL-101 infusion, the intensity is the same but the drug relationship became related during the active phase of the study.

Summary statistics (mean, median, SD and range as appropriate) will be presented for vital signs, ECG parameters and clinical laboratory tests at each assessment along with change from baseline. For laboratory data, abnormal values will be flagged in the data listings. Shift tables will be presented of the number and percentage of subjects with low, normal or high values and normal or abnormal examinations at each visit and overall.

9.7 Pharmacokinetic Analysis

Pharmacokinetic blood samples will be collected before each Busulfan dose is administered, at the end of Busulfan infusion, +1, 2, 4, 8, and 13 hours post-infusion (+/- 15 minutes).

The following Busulfan PK parameters will be summarized. In addition, graphical presentation of average concentration (using original and log scale) will be presented over time.

C_{max}	Maximum plasma concentration achieved
T_{max}	Time to C_{max} (peak exposure)
AUC_{0-t}	The area under the plasma concentration-time curve up to the last quantifiable concentration (LOQ; limit of quantitation) from time of administration (t=0) up to the selected interval after the injection, calculated by the linear trapezoidal method
AUC0-13	The area under the plasma concentration-time curve during the first 13 hours post injection, calculated by the linear trapezoidal method
$AUC_{0-\infty}$	The area under the plasma concentration-time curve extrapolated to infinity, calculated as: AUC0-∞ = AUC0-t + Clast/λz, where Clast is the last measurable (>LOQ) concentration.
λ_z	Elimination rate constant determined by linear regression of the terminal points of the ln-linear plasma concentration-time curve
t1/2	Terminal elimination half-life, defined as 0.693/λz

10 DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS

The Investigator must maintain the primary records (i.e. source documents) of each subject's data at all times. The Investigator will maintain a confidential subject identification list that allows the unambiguous identification of each subject. All study related documents must be kept until notified by the Sponsor.

The medical experts, study monitors, auditors, and health authority inspectors (or their agents) will be given direct access to source data and documentation (e.g. medical charts/records, laboratory test results, printouts, videotapes) for source data verification, provided that subject confidentiality is maintained in accordance with local requirements.

Authorized personnel from external CAs, Clinical Research Organization (CROs) and Quality Assurance personnel authorized by the Sponsor may carry out inspections and audits. The purpose of an audit is to ensure that ethical, regulatory and quality requirements are fulfilled in all studies performed by the Sponsor. Auditors and inspectors must have direct access to the study documents and site facilities as specified in Section 11.5, and to any other locations used for the purposes of the study in question (e.g. laboratories).

In the event of the site being notified directly of a regulatory inspection, the Investigator must notify the Sponsor's representative as soon as possible, to assist with preparations for the inspection.

11 QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Protocol Amendments

11.1.1 Protocol Amendments

No changes from the final approved (signed) protocol will be initiated without the prior written approval or favorable opinion of a written amendment by the IEC/IRB and CAs, except when this is necessary to eliminate immediate safety concerns to the subjects or when the change involves only logistics or administration. The principal Investigator and the Sponsor will sign the protocol amendment.

In the event that an amendment to this protocol is required, it will be classified into one of the following three categories:

Administrational changes are those that are not considered 'substantial' (e.g. administrative changes) and as such are not required to be notified to the IEC/IRB or CAs but are contained in the protocol under a subsequent notification of a substantial amendment.

Substantial Amendments are those considered 'substantial' to the conduct of the clinical study where they are likely to have a significant impact on:

- the safety or physical or mental integrity of the subjects,
- the scientific value of the study,
- the conduct or management of the study, or
- the quality or safety of the protocol treatment used in the study.

Substantial amendments must be notified to the IEC/IRB and CAs. Prior to implementation, documented approval must be received from the IEC/IRB and either explicit approval or no raise of grounds of non-acceptance during the regulatory review period (country specific) must be received from the relevant CAs.

Urgent Amendments are those that require urgent safety measures to protect the study subjects from immediate hazard and as such may be implemented immediately by the Sponsor with subsequent IEC/IRB and CAs notification, forthwith.

11.1.2 Protocol Deviations, Violations, and Exceptions

A protocol deviation concerns non-compliance with protocol-specific study procedures or schedules that do not involve inclusion/exclusion criteria, primary objective evaluation criteria, and/or GCP guidelines. Such deviations are considered to be minor and do not impact the study.

A protocol violation is any clinically significant divergence from the protocol, i.e. non-compliance by the subject, the Investigator, or the Sponsor with protocol-specific inclusion/exclusion criteria, primary objective evaluation criteria, and/or GCP guidelines. Protocol violations will be identified and recorded, by study center personnel, in the eCRF.

As a matter of policy, the Sponsor will not grant any exemptions from the protocol-specific entry criteria in order to allow subjects to enter a study. If, under extraordinary circumstances, such an action is considered ethically, medically and scientifically justified for a particular subject, prior approval from the Sponsor and the responsible IEC/IRB is required before the subject will be allowed to enter the study. If staff at an investigational center learn that a subject who did not meet the protocol eligibility criteria was entered in a study (a protocol violation), they must inform the Sponsor immediately. Such subjects will be withdrawn from the study,

except in exceptional cases following review and written approval by the Sponsor and the responsible IEC/IRB.

11.2 Dissemination of Information to Study Personnel

The Investigator is responsible for giving information on the study to all staff members involved in the study or in any element of subject management, both before starting any study procedures and during the course of the study (e.g. when new staff become involved). The Investigator must ensure that all study staff members are qualified by education, experience and training to carry out their specific responsibilities. These study staff members must be listed on the study center authorization form, which includes a clear description of each staff member's responsibilities. This list must be updated throughout the study, as necessary.

The study monitor is responsible for explaining the protocol to all study staff, including the Investigator, and for ensuring their compliance with the protocol. Additional information will be made available during the study when new staff become involved in the study and as otherwise agreed with either the Investigator or the study monitor.

11.3 Study Monitoring

The Investigator is responsible for the validity of all data collected at the site.

The Sponsor is responsible for monitoring this data to verify that the rights and well-being of subjects are protected, that study data are accurate (complete and verifiable to source data) and that the study is being conducted in compliance with the protocol, GCP and regulatory requirements.

11.4 Routine Monitoring

To ensure compliance with GCP, the study monitor or representative is responsible for ensuring that the study is conducted according to applicable standard operating procedures (SOPs), the protocol, and other written instructions and regulatory guidelines.

Sponsor assigned monitors will conduct regular site visits according to the monitoring plan. The Investigator will allow direct access to all relevant files (for all subjects) and clinical study supplies (dispensing and storage areas) for the purpose of verifying entries made in the eCRF, and will assist with the monitor's activities, if requested. Adequate time and space for monitoring visits should be made available by the Investigator.

The site must complete the eCRFs according to the Sponsor's monitoring manual with respect to all visits by the subjects and on an ongoing basis (within a maximum 5 days) to enable their regular review by the study monitor, both remotely via the internet and during site visits. The study monitors will use functions of the EDC system to address any queries that arise while reviewing the data entered by study site personnel, in a timely manner.

Whenever a subject's name is revealed on a document required by the Sponsor (e.g. laboratory print outs) the name must be blacked out permanently by the site personnel leaving only the initials visible and annotated with the subject's number as identification.

As part of the supervision of study progress, other Sponsor personnel may, upon request, accompany the study monitor on visits to the study centre. The Investigator and assisting staff must agree to cooperate with the study monitor to resolve any problems, errors, or possible misunderstandings concerning the findings detected in the course of these monitoring visits.

11.5 Audit and Inspection

Authorised personnel from external CAs and the Sponsor's authorised Quality Assurance personnel or nominated representative (CRO) may carry out inspections and audits.

11.6 Data Quality Assurance

Monitored eCRFs transferred from the investigational site to the assigned Data Management group will be reviewed (secondary monitoring) for completeness, consistency, legibility and protocol compliance.

Reasons should be given on the relevant eCRF for any missing data and other protocol deviations. Any electronic queries and items not adequately explained will require additional electronic manual queries to be raised to the Investigator by the monitor for clarification/correction. The Investigator must ensure that queries are dealt with promptly. All data changes and clarifications can be viewed in the audit trail function of the eCRF.

The handling of data, including data quality assurance, will comply with regulatory guidelines (e.g., ICH and GCP) and the Sponsor's SOPs and working instructions. Data management and control processes specific to this study will be described in a data management plan. All steps and actions taken regarding data management and quality assurance will be documented in a data handling report.

The Sponsor will implement edit checks on the eCRF to enforce data entry guidelines, data consistency, and compliance to the protocol and regulatory requirements. The Study centre coordinator(s) will be responsible for entering study data on the eCRFs. Data management will track eCRFs and review them for completeness, the presence of mandatory values, consistency, and dated electronic signatures. In addition to checking for source data verification flags, data management will electronically attach data clarification queries directly onto the eCRFs during the review process to ensure data quality. Once study centre personnel have provided acceptable responses to the queries and implemented the changes on the eCRFs, data management will close the queries with the appropriate resolution status.

During the study the database will be locked for the interim analysis (first five subjects at the 6-month visit), 12-month (primary) analysis and the 24-month analysis at the end of study. The data will be released for reporting and statistical analysis.

12 ETHICS

12.1 Compliance with Good Clinical Practice and Ethical Considerations

This study will be conducted in compliance with IEC/IRB, local regulation for informed consent/assent, the Declaration of Helsinki and ICH GCP Guidelines.

The EDC system will comply with FDA, 21 CFR Part 11, Electronic Records, Electronic Signatures, and FDA, Guidance for Industry: Computerized Systems Used in Clinical Trials.

In addition, this study will adhere to all local regulatory requirements.

Before initiating a study, the Investigator/institution must have obtained written and dated approval/favorable opinion from the IEC/IRB for the study protocol/amendment(s), written informed consent forms, any consent form updates, subject emergency study contact cards, subject recruitment procedures (e.g. advertisements), any written information to be provided to subjects and a statement from the IEC/IRB that they comply with GCP requirements. The IEC/IRB approval must identify the protocol version as well as the documents reviewed.

After IEC/IRB approval, changes will require a formal amendment. Once the study has started, amendments should only be made in exceptional circumstances. Changes that do not affect subject safety or data integrity are classified as administrative changes and generally do not require ethical approval. If ethically relevant aspects are concerned, the IEC/IRB must be informed and, if necessary, approval sought prior to implementation. Ethical approval of administrative changes will be obtained if required by local/site IEC/IRB.

12.2 Informed Consent

Prior to study entry, the Investigator, or a person designated by the Investigator, will explain the nature, purpose, benefits and risks of participation in the study to each subject, the subject's parents/legal guardians or an impartial witness. The Sponsor will provide sample informed consent/assent forms for the parents/legal guardians and subjects to read and sign. The final version controlled form must be agreed by the Sponsor, and the IEC/IRB and must contain all elements included in the sample form, in language readily understood by the subject. A signed and dated informed consent form will be obtained from each parent/legal guardian and a signed and dated assent form will be obtained from each subject (according to local regulations) before any study specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained, according to local IEC/IRB requirements. Sufficient time must be allowed to discuss any questions raised by the subject and parents/legal guardians.

The Investigator will keep the original consent/assent forms and copies will be given to the subjects and their parents/legal guardians. It will also be explained to the subjects and their parents/legal guardians that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment.

The consent/assent form(s) may need to be revised during the study should important new information become available that may be relevant to the safety of the subject or as a result of protocol amendments. In this instance, approval should always be given by the IEC/IRB. It is the Investigator's responsibility to ensure that all subjects and the subject's parents/legal guardians (including those subsequently entered into the study and those currently in the study) sign the amended form. This is documented in the same way as previously described. Subjects

(and their parents/legal guardians) who have completed the study should be informed of any new information that may impact their welfare/well-being.

The Investigator should, with the consent of the subject, inform the subject's primary physician about their participation in the clinical study.

12.3 Health Authorities and Independent Ethics Committees/Institutional Review Boards

As required by local regulations, the Sponsor's Regulatory Affairs group will ensure all legal regulatory aspects are covered, and obtain approval from the appropriate regulatory bodies prior to study initiation in regions where such an approval is required.

Before this study starts, the protocol will be submitted to the national/local health authorities and to each IEC/IRB for review. As required, the study will not start [at a given centre] before the IEC/IRB and health authority (where applicable) for the centre give written approval or a favourable opinion or does not raise any grounds of non-acceptance within the review timeframe.

12.4 Confidentiality Regarding Study Subjects

The Investigator must assure that the privacy of the subjects, including their personal identity and all personal medical information, will be maintained at all times. In eCRFs and other documents or image materials submitted to the Sponsor, the subjects will be identified not by their names, but by an identification code (e.g. initials and identification number).

Personal medical information may be reviewed for the purposes of verifying data recorded in the eCRF. This review may be conducted by the study monitor, properly authorized persons acting on behalf of the Sponsor, the quality assurance unit, or regulatory authorities. Personal medical information will always be treated as confidential.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Recording of Study Data

In compliance with GCP, all medical records/medical notes, etc., should be clearly marked and permit easy identification of a subject's participation in the specified clinical study. The Investigator must record all data relative to protocol procedures, study product administration, laboratory data, safety data and efficacy ratings in the eCRFs provided for the study. The Investigator, by completing the signature log, may formally designate authority to complete eCRFs to appropriately qualified staff having certified user access to the eCRF. Questionnaires completed by the subjects will be printed out.

The Investigator must, as a minimum, provide an electronic signature (e-signature) for each case report book so as to attest to the accuracy and completeness of all the data. If any changes are made to the eCRF once a form has been locked and electronically signed, the Investigator will be required to approve an additional e-signature indicating his/her agreement with any new information or changes to the eCRF. All corrections in the eCRF will be automatically tracked and a reason for any change is always required. In the eCRF, the audit trail function will allow the changes made to be viewed on each item entered.

13.2 Data Management

Electronic Data Capture will be utilized to collect all subject data. Each site is required to have a computer and internet connection available for the site entry of clinical data. All entries in the eCRF will be made under the electronic signature of the person performing the action. This electronic signature consists of an individual and confidential username and password combination. It is declared to be the legally binding equivalent of a handwritten signature. Only Sponsor authorized users will have access to the eCRF, as appropriate to their study responsibilities. Users must have successfully completed software application training prior to entering data in the eCRF.

Data management will be performed by a CRO. All data management procedures will be carried out in accordance with the contracted CRO's SOPs. Prior to data being received in-house by the assigned CRO, they will be monitored at the Investigator site, (for further details please see Section 11.3). The eCRF and other data documentation removed from the Investigator site(s) will be tracked by the CRO and the monitor.

The Sponsor will ensure that an appropriate eCRF is developed to capture the data accurately, and that suitable queries are raised to resolve any missing or inconsistent data. The Investigator will receive data from the clinical study in an electronic format (PDF files) that will be an exact copy of the eCRF and will include the full audit trail, for archiving purposes and future reference.

Any queries generated during the data management process will also be tracked by the contracted data management CRO/will be raised within the EDC system. It is the central study monitor's responsibility to ensure that all queries are resolved by the relevant parties.

The Sponsor will also ensure that SAE data collected in the eCRF are consistent with information provided to the Sponsor's pharmacovigilance department (and vice versa).

The coding of an AE, medical history and concomitant medication terms will be performed by the contracted CRO, directed by the Medical Monitor, and reviewed and approved by the Sponsor.

Concomitant medications will be coded using WHO drug dictionary and AEs/medical history terms will be coded using Medical Dictionary for Regulatory Activities (MedDRA).

13.2.1 Investigator Responsibilities

All records related to the study (i.e. source data, source documents, CRFs (see Section 10), copies of protocols and protocol amendments, drug accountability forms, correspondence, subject identification lists, signed informed consent forms, and other essential documents) must be retained until the Sponsor notifies the institution, in writing, that records may be destroyed.

If the Sponsor has not provided written notification of records destruction after 10 years from study completion (or earlier in the case of an institution closing), and the institution determines the study record retention is unduly burdensome, the institution may submit a written request to the Sponsor at least 60 days before the planned disposition of the study records. No study document or image (e.g. scan, radiograph, ECG tracing) should be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, advance written notice will be given to the Sponsor.

13.2.2 Sponsor Responsibilities

The Sponsor will be responsible for the processing and quality control of the data. Data management and filing will be carried out as described in the Sponsor's SOPs for clinical studies.

If data management and filing for this study are delegated to a contract organization, these functions will be carried out as described in the SOPs for clinical studies at that organization. These SOPs will be reviewed by the Sponsor prior to the start of data management and filing activities.

The original CRFs will be archived by the Sponsor for 30 years (according with gene therapy traceability requirement). If eCRFs are used in the study, electronic images will be archived by the Sponsor. Centre specific eCRF images will be sent to the study centre for archiving.

13.3 Record Archiving and Retention

During the pre-study and initiation visits, the monitor must ensure that the archiving facilities are adequate and the archiving/retention responsibilities of the Investigator have been discussed.

Study documents should be retained for at least 2 years counted from the last approval of a marketing application in an ICH region and until there are no pending or planned marketing applications in an ICH region (that is at least 15 years), or at least 2 years have elapsed since the formal discontinuation of clinical development of the product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the Sponsor. The Investigator should take measures to prevent any accidental or premature destruction of these documents. The final archiving arrangements will be confirmed by the monitor when closing out the site. The Sponsor will inform the Investigator, in writing, as to when these documents no longer need to be retained.

If the principal Investigator relocates or retires, or otherwise withdraws his/her responsibility for the maintenance and retention of study documents, the Sponsor must be notified (preferably in writing) so that adequate provision can be made for their future maintenance and retention.

14 FINANCING AND INSURANCE

14.1 Contractual and Financial Details

The Investigator (and/or, as appropriate, the hospital administrative representative) and the Sponsor will sign a clinical study agreement prior to the start of the study, outlining the overall responsibilities of the Sponsor and Investigator relative to the study. Financial remuneration will cover the cost per subject included, based on the calculated costs of performing the study assessments in accordance with the protocol, and the specified terms of payment will be described in the contract. The contract should describe whether the costs of pharmacy, laboratory and other protocol required services are being paid directly or indirectly.

Financial Disclosure Statements will need to be completed, as requested by FDA 21 CFR Part 54.

14.2 Insurance, Indemnity and Compensation

The Sponsor will provide Product Liability insurance for all subjects included in the clinical study. Where required, a hospital specific indemnity agreement will be used.

The study is covered under a liability insurance policy. The certificate of insurance and essential information about the insurance coverage can be provided upon request.

15 REPORTING AND PUBLICATION OF RESULTS

15.1 Publication Policy

Publication of the results of this study will be governed by the publication policies of the funding institutes. Any presentation, abstract, or manuscript must be made available for review by the NIH supporters prior to submission.

This clinical study will be registered on the ClinicalTrials.gov website.

15.2 Clinical Study Report

A final clinical study report (CSR) will be prepared according to the ICH guideline on structure and contents of CSRs. A final CSR will be prepared where any subject has signed informed consent, regardless of whether the study is completed or prematurely terminated. Where appropriate an abbreviated report may be prepared. The CSR will be in compliance with any applicable regulatory requirements, national laws in force and will be in English.

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17 APPENDICES

Appendix 1: OTL-101 Infusion Procedure and Dosing Forms

Required Equipment

Thaw:

- Water bath or plasma thawing device
- Thermometer
- Sterile water for thawing
- Sterile plastic zip-lock bag
- 70% isopropanol

Infusion:

- Syringes: 20 and/or 30mL
- 3-way stopcock
- Infusion set

Additional equipment:

- Insulated cryogenic gloves
- PPE (Personal Protective Equipment): Safety glasses, gloves and Disposable gown
- Emergency medical equipment:

  OTL-101 is an investigational product, and there is a possible risk of anaphylaxis. Emergency medical equipment (including emergency medications) must be available during the infusion in case the subject has an allergic response, severe hypotensive crisis, or any other reaction to the infusion.

Infusion Procedure

General

- Obtain and review the Dose and Infusion Volume Calculation Worksheet which was completed prior to Busulfan administration,
- Prepare the water bath or plasma-thawing device at least 1 hour prior to thawing. Verify the temperature is stable at 37ºC (acceptable range 36 to 39ºC) prior to removing the OTL-101 container from the dry shipper,
- Pre-medicate the subject with acetaminophen and diphenhydramine 30 minutes to 1 hour prior to infusion. These medications may be repeated every 3 to 4 hours as needed.

- Prior to beginning the infusion and no more than 1 hour prior to the infusion, record the subject's vital signs on the Infusion Record.
- The infusion will be via an indwelling latex free 18 gauge/4 French catheter or other equivalent IV access. Ensure the subject has a patent catheter and that it has a female luer or other access capable of a three-way stopcock connection prior to thawing the product. No inline filter or pump should be used,
- If necessary, start a 0.9% USP normal saline infusion to keep the IV access open until the OTL-101 Product is ready for infusion,
- Collect any blood samples prior to the start of infusion of the OTL-101 product,
- The nurse or physician must be present during the infusion.

Infusion

- Attach a 20 or 30 mL syringe and an infusion set to two ports of a 3-way stopcock. Disconnect the NS line and attach the remaining port to the IV catheter,
- Prior to thawing, inspect the OTL-101 Product bag for cracks, broken seals or damage. Two individuals must verify the subject information on each OTL-101 Product bag with the subject ID bracelet. Any discrepancy must be investigated prior to thawing the product (be sure to replace the OTL-101 Product bag in the LN2 dry shipper until the discrepancy is resolved),
- Thawing of the product bags will take approximately 2 to 5 minutes. Place the OTL-101 Product bag into a sterile overwrap container and then place in the plasma thawing device or water bath. The OTL-101 Product is ready to infuse when the last ice crystals dissolve. If there are multiple bags, each bag must be thawed separately. Only one OTL-101 product bag may be infused per hour,
- **Record the OTL-101 Product thaw start time in the patient medical record and e-CRF,**
- Prior to removing the thawed OTL-101 Product bag from the sterile overwrap container, inspect for evidence of leakage or gross abnormalities. Refer to the institution emergency salvage procedures when necessary and use medical judgement to determine if the product bag should not be infused for any reason,
- Remove the thawed OTL-101 Product bag from the sterile overwrap container and insert the spike end of the infusion set into the port of the OTL-101 Product bag and open the clamp,
- Using the attached syringe, withdraw the OTL-101 Product into the syringe. The air in the tubing line may be returned to the product bag to facilitate volume transfer,
- Rotate the stopcock to open the path between the syringe and the subject's IV access and slowly begin to infuse the cells.

- **Record the infusion start time in the patient medical record and e-CRF,**
- The cell product should be infused over 5 to 15 minutes and within 30 minutes after it has been thawed,
- Rotate the stopcock to close the path to the IV and clamp the infusion tubing.
- If applicable, rinse the OTL-101 product bag by either gravity transfer of approximately 10 mL of normal saline or by replacing the syringe with a new syringe containing approximately 10 mL of normal saline and rotating the stopcock to open the path between the syringe and the OTL-101 Product bag. Gently rotate the OTL-101 product bag to rinse. Withdraw the rinse into the syringe and infuse the rinse as described above,

  NOTE: If only a portion of the product is to be infused, do not rinse the bag after that volume is withdrawn and infused to the subject.
- **When the infusion is complete, record the stop time in the patient medical record and e-CRF,**
- If applicable, begin thawing the next OTL-101 Product bag when the prior infusion is complete. Do not infuse more than one OTL-101 product bag per hour.

Post Infusion

- Resume the normal saline infusion for 30 minutes to ensure that no product remains in the IV catheter tubing,
- Record the vital signs (blood pressure, pulse, and temperature) after all infusion bags are completed,
- Continue to record the vital signs every 30 minutes according to the study center procedures for a minimum of 2 hours and until stable after the infusions are completed. Record in the patient medical record and e-CRF,
- Discard the OTL-101 Product bags and infusion tubing in biohazard waste.

Final Product Dose and Infusion Volume Calculation – Single Product Bag

Subject Number / PIN	**Medical Record Number**
ADA -	

1. Obtain the Final Product Certificate of Analysis (CofA) which contains the values for the FP Total $CD34^+$ cells and the weight at harvest admission. Record those values below. Calculate and record the FP $CD34^+$ cells per kg as follows: Divide the FP Total $CD34^+$ cells by the Weight.

	x 10^6	÷		=		10^6/ kg
FP Total $CD34^+$ cells		Weight at Harvest		**FP $CD34^+$ cells / kg**		

2. Select course of action based on the following criteria:

○ The **FP $CD34^+$ cells per kg** is less than 2.0x10^6 $CD34^+$/kg. An additional FP manufacturing process will be required to obtain the minimum acceptable dose

○ The **FP $CD34^+$ cells per kg** is greater than 2.0x10^6 $CD34^+$/kg and less than 20.0x10^6 $CD34^+$/kg. Thaw and infuse the entire FP volume

○ The **FP $CD34^+$ cells per kg** is greater than 20.0x10^6 $CD34^+$/kg, calculate the volume to infuse as follows and do not rinse the OTL-101 bag after infusion:

Obtain the FP CofA which contains the values for the FP $CD34^+$ cells/mL and the weight at harvest admission
Calculate the infusion volume by multiplying 20.0x10^6 $CD34^+$/kg by the weight and then dividing by the FP $CD34^+$ cells/mL

20.0x10^6 $CD34^+$/kg	x	kg	=			x10^6 $CD34^+$
		Weight at Harvest		Total $CD34^+$ to Infuse		

	x10^6 $CD34^+$	÷		x$10^6$$CD34^+$/mL	=	mL
Total $CD34^+$ to Infuse		FP $CD34^+$cells/mL				Infusion Volume

Completed by: (print & sign) _____ Date: _____

Verified by: (print & sign) _____ Date: _____

Final Product Dose and Infusion Volume Calculation – Multi Product Bag

Subject Number / PIN	**Medical Record Number**
ADA -	

1. Obtain the Final Product Certificate of Analysis (CofA) which contains the values for the FP Total $CD34^+$ cells and the subject weight at harvest admission. Record the values below and calculate the FP CD34+ cells per kg as follows: Divide the FP Total CD34+ cells by the Weight at Harvest.

Bag 1: _____ x10^6 ÷ _____ = _____ x10^6/kg
FP Total $CD34^+$ cells | Weight at Harvest | Bag 1 CD34+ cells / kg

Bag 2 _____ x10^6 ÷ _____ = _____ x10^6/kg
FP Total $CD34^+$ cells | Weight at Harvest | Bag 2 CD34+ cells / kg

2. Calculate the sum and record the Combined FP $CD34^+$ cells per kg as follows:

_____ x 10^6/kg ÷ _____ x 10^6/kg = _____ x 10^6/kg
Bag 1 $CD34^+$ cells/kg | Bag 2 $CD34^+$ cells/kg | Combined $CD34^+$ cells/kg

3. Select course of action based on the following criteria:

○ The Combined **$CD34^+$ cells per kg** is less than 2.0x10^6 $CD34^+$/kg. An additional final product manufacturing process will be required to obtain the minimum acceptable dose

○ The Combined **$CD34^+$ cells per kg** is greater than 2.0x10^6 $CD34^+$/kg and less than 20.0x10^6 $CD34^+$/kg. Thaw and infuse both FP bags. Do not infuse more than one FP bag per hour.

○ The Combined **$CD34^+$ cells per kg** is greater than 20.0x10^6 $CD34^+$/kg, calculate the volume to infuse as follows. Do not infuse more than one FP bag per hour and do not rinse the second OTL-101 bag after infusion:

- Identify the FP bag contains a smaller FP Total $CD34^+$ cell number. This bag will be partially infused. If both bags contain the same FP $CD34^+$ cell number, FP Bag 2 will be partially infused.
- Calculate the $CD34^+$ cell number that will be required from the partially infused bag 2 as follows:

20.0x10^6 $CD34^+$/kg x _____ = _____ x 10^6
Weight at 2nd Harvest | Total $CD34^+$ cells to infuse

- Calculate the $CD34^+$ cell number and volume of the 2nd FP bag to infuse as follows:

	x 10^6	-		x 10^6	=	x 10^6
Total $CD34^+$ cells to infuse		FP Total $CD34^+$ cells (Bag 1)			FP Bag 2 $CD34^+$ cells to infuse	

	÷		=	mL	
FP Bag 2 $CD34^+$ cells to infuse		FP Bag 2 $CD34^+$cells/mL		FP Bag 2 volume to infuse	

Completed by: (print & sign) _____ Date: _____

Verified by: (print & sign) _____ Date: _____

Appendix 2: Global Investigational Product Accountability Log

Study #: OTL-101-4	**Site #: UCLA**	[redacted]	**Investigational Product:** *(name, dosage, form, etc.)*:

PIN_____

		GMP Lab.			**Stem Cell Lab**			**UCLA Site**				
Subject DoB	**Subject Medical Record #**	**Dispensed By (Initials)**	**Date Mm/dd/yyyy**	**Time**	**Dispensed By (Initials)**	**Date**	**Time**	**Dispensed By (Initials)**	**Date**	**Time**	**Status**	**Verification CRA (Initials & Date)**
			Click here to enter a date.			Click here to enter a date.			Click here to enter a date.		☐ Dispensed ☐ Unused*	
			Click here to enter a date.			Click here to enter a date.			Click here to enter a date.		☐ Used ☐ Unused*	
			Click here to enter a date.			Click here to enter a date.			Click here to enter a date.		☐ Used ☐ Unused*	

*if "unused" is reported, explanations are required:

Subject not eligible	☐	Inconsistent paperwork	☐	Inconsistent information on subject ID	☐
Damaged outer packaging	☐	Storage issue	☐	Other: _____	☐

Global Investigational Product Accountability Log (Continued)

Study #: OTL-101-4	**Site #: UCLA**	[redacted]	**Investigational Product:** *(name, dosage, form, etc.)*:

*If "unused", please record the follow-up actions:

Product discarded	☐	Product returned to stem cell lab.	☐	No further action	☐
Product returned to GMP lab.	☐	Product kept at the clinic	☐	Other:_____	☐

Comments:

Final Accountability

Investigator: (Print Name) _____ Signature _____ Date _____

Monitor: (Print Name) _____ Signature _____ Date _____

Appendix 3: Lanksy and Karnofsky Scales

Karnofsky Scale (recipient age ≥ 16 years)	Karnofsky Scale (recipient age < 16 years)
Able to carry on normal activity: no special care is needed	Able to carry on normal activity: no special care is needed

100	Normal, no complaints, no evidence of disease	100	Fully active
90	Able to varry on normal activity	90	Minor restrictions in physically strenous play
80	Normal activity with effort	80	Restricted in strenusou play, tires more easily, otherwise active
Unable to work, able to live at home, cares for most personal needs, a varying amount of assistance is needed		Mild to moderate restrictions	
70	Care for self, unable to carry on normal actvitiy or to do active work	70	Both greater restrictions of, and less time spent in active play
60	Requires occasional assistance but is able to care for most needs	60	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Requires considerable assistance and frequent medical care	50	Considerable assistance required for any active play, fully able to engage in quiet play
Unable to care for self, requires equivalent of institutional or hospital care, disease may be progressing rapdily		Moderate to severe restriction	
40	Disabled, requires special care and assistance	40	Able to initiate quiet activities
30	Severely disabled, hospitalization indicated, although death not imminent	30	Needs considerable assistance for quiet activity
20	Very sick, hospitalization necessary	20	Limited to very passive activity initiated by other (e.g., TV)
10	Moribund, fatal process progressing rapdily	10	Completely disabled, not even passive play

The Karnofsky/Lansky Scale should be recorded in the eCRF according to the schedule of assessments in Table 1 and Table 2.

Appendix 4: DAIDS Table of Toxicity for Adverse Events

Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events

Version 2.0
November 2014

Division of AIDS
National Institute of Allergy and Infectious Diseases
National Institutes of Health
US Department of Health and Human Services

GLOSSARY AND ACRONYMS

AE	Adverse event; Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.
ALT (SGPT)	Alanine aminotransferase (serum glutamic pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase (serum glutamic-oxaloacetic transaminase)
AV	Atroventricular
Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer or movement, continence, and feeding. Young Children Activities that are age and culturally appropriate, such as feeding one's self with culturally appropriate eating implements.
BMI z-score	Body mass index z- score; A body reference norm. Specifically, the number of standard deviations a participant's BMI differs from the average BMI for their age, sex, and ethnicity.
BMD t-score	Bone mineral density t-score; The number of standard deviations above or below the mean bone mineral density of a healthy 30 year old adult of the same sex and ethnicity as the participant.
BMD z-score	Bone mineral density z-score; The number of standard deviations a participant's BMD differs from the average BMD for their age, sex, and ethnicity.
BPAP	Bilevel positive airway pressure; A mode used during noninvasive positive pressure ventilation.
Chemical Pregnancy	A pregnancy in which a positive pregnancy test is followed by a negative pregnancy test without evidence of a clinical pregnancy loss.
CNS	Central nervous system
CPAP	Continuous positive airway pressure
DAERS	DAIDS Adverse Experience Reporting System; An internet-based system developed for clinical research sites to report Expedited Adverse Events (EAEs) to DAIDS. It facilitates timely EAE report submission and serves as a centralized location for accessing and processing EAE information for reporting purposes
Disability	A substantial disruption of a person's ability to conduct normal life functions.
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
Hospitalization	Does not include the following hospital admissions: under 24 hours, unrelated to an adverse event (e.g., for labor and delivery, cosmetic surgery, social or administrative for temporary placement [for lack of a place to sleep]), protocol-specified, and for diagnosis or therapy of a condition that existed before the receipt of a study agent and which has not increased in severity or frequency
INR	International normalized ratio

Intervention	Medical, surgical, or other procedures recommended or provided by a healthcare professional for the treatment of an adverse event
IV	Intravenous
IVIG	Intravenous immune globulin
LD	Low density lipoprotein
LLN	Lower limit of normal
Life-threatening AE	Any adverse event that places the participant, in the view of the investigator, at immediate risk of death from the reaction when it occurred (i.e., it does not include a reaction that would have caused death if it had occurred in a more severe form).
NA	Not applicable
Participant ID	The identification number assigned to a study participant which is used to track study-related documentation, including any reported AEs.
PR Interval	The interval between the beginning of the P wave and the beginning of the QRS complex of an electrocardiogram that represents the time between the beginning of the contraction of the atria and the beginning of the contraction of the ventricles.
PT	Prothrombin time
PTT	Partial thromboplastin time
QTc Interval	The measure of time between the onset of ventricular depolarization and completion of ventricular repolarization corrected for ventricular rate.
RBC	Red blood cell
SI	Standard international unit
ULN	Upper limit of normal
Usual Social & Functional Activities	Activities which adults and children perform on a routine basis and those which are part of regular activities of daily living, for example: Adults Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, or pursuing a hobby. Young Children Activities that are age and culturally appropriate, such as social interactions, play activities, or learning tasks.
WBC	White blood cell
WHO	World Health Organization
WNL	Within normal limits

INTRODUCTION

The Division of AIDS (DAIDS) oversees clinical trials throughout the world which it sponsors and supports. The clinical trials evaluate the safety and efficacy of therapeutic products, vaccines, and other preventive modalities. Adverse event (AE) data collected during these clinical trials form the basis for subsequent safety and efficacy analyses of pharmaceutical products and medical devices. Incorrect and inconsistent AE severity grading can lead to inaccurate data analyses and interpretation, which in turn can impact the safety and well-being of clinical trial participants and future patients using pharmaceutical products.

The DAIDS AE grading table is a shared tool for assessing the severity of AEs (including clinical and laboratory abnormalities) in participants enrolled in clinical trials. Over the years as scientific knowledge and experience have expanded, revisions to the DAIDS AE grading table have become necessary.

The *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse*

Events, Version 2.0 replaces the grading table published in 2004 and updated in 2009. In version 2.0, AEs not previously included, but which now are deemed medically important events, are included while other AEs have been removed. Some AE severity grading descriptions have been revised to more appropriately reflect the presentation of these events in clinical settings and their impact on clinical trials. For example, DAIDS performed an extensive literature search and reviews of select DAIDS clinical trial data in revising certain hematology parameters (i.e., hemoglobin, white cell counts, and absolute neutrophil counts). DAIDS also took into consideration the U.S. Food and Drug Administration's guidance regarding the use of local laboratory reference values and ethnic differences among certain healthy adolescent and adult populations in defining parameter limits. Finally, the revised DAIDS AE grading table also contains an updated glossary and acronyms section, expanded instructions for use section, and an appendix that provides more age-specific information for an AE of concern to DAIDS.

DAIDS is grateful to the DAIDS Grading Table Working Group, numerous government and non-government affiliated medical subject matter experts and reviewers who were instrumental in the revision of the DAIDS AE grading table.

GENERAL CONSIDERATIONS

The *Division of AIDS* (*DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0* consists of parameters, or AEs, with severity grading guidance that are to be used in DAIDS clinical trials for safety data reporting to maintain accuracy and consistency in the evaluation of AEs. The term "severe" is not the same as the term "serious" in classifying AEs. The severity of a specific event describes its intensity, and it is the intensity which is graded. Seriousness, which is not graded, relates to an outcome of an AE and is a regulatory definition.

Clinical sites are encouraged to report parameters in the DAIDS AE grading table as they are written to maintain data consistency across clinical trials. However, since some parameters can be reported with more specificity, clinical sites are encouraged to report parameters that convey

additional clinical information. For example, diarrhea could be reported as neonatal diarrhea; seizures, as febrile seizures; and pain, as jaw pain.

The DAIDS AE grading table provides an AE severity grading scale ranging from grades 1 to 5 with descriptions for each AE based on the following general guidelines:

- Grade 1 indicates a mild event
- Grade 2 indicates a moderate event
- Grade 3 indicates a severe event
- Grade 4 indicates a potentially life-threatening event
- Grade 5 indicates death (*Note*: This grade is not specifically listed on each page of the grading table).

Other points to consider include:

- Use parameters defined by age and sex values as applicable.
- Male and female sex are defined at birth.
- Unless noted, laboratory values are for term neonates. Preterm neonates should be assessed using local laboratory normal ranges.
- Where applicable, Standard International (SI) units are included in italics.

SELECTING AND REPORTING A PRIMARY AE TERM

When selecting a primary AE term to report, sites should select the term that best describes what occurred to the participant. For example, a participant may present with itching, urticaria, flushing, angioedema of the face, and dyspnea. If the underlying diagnosis is determined to be an acute allergic reaction, sites should report "Acute Allergic Reaction" as the primary AE term.

Primary AE terms should be reported using the DAIDS Adverse Experience Reporting System (DAERS) only if they meet expedited reporting criteria. However, all primary AE terms should be reported using protocol-specific case report forms (CRFs). Because the reported information is stored in different databases (i.e., safety and clinical), sites should report primary AE terms using the same terminology for data consistency.

When reporting using DAERS, other clinically significant events associated with a primary AE term that more fully describe the nature, severity, or complications of the primary AE term should be entered in the "Other Events" section. However, the severity grade for these events must be lower than or equal to the severity grade of the primary AE term. In the example above, dyspnea and angioedema of the face may be entered in the "Other Events" section, because they are more descriptive and provide additional information on the severity of the acute allergic reaction. However, their severity grades must be lower than or equal to the severity grade of the primary AE term of "Acute Allergic Reaction".

Differences exist in the reporting and recording of information (e.g., signs and symptoms, clinically significant events) in DAERS and CRFs. Therefore, sites should refer to their protocols and CRF requirements for further instructions.

GRADING ADULT AND PEDIATRIC AES

When a single parameter is not appropriate for grading an AE in both adult and pediatric populations, separate parameters with specified age ranges are provided. If no distinction between adult and pediatric populations has been made, the listed parameter should be used for grading an AE in both populations.

REPORTING PREGNANCY OUTCOMES

In the *Pregnancy, Puerperium, and Perinatal* section, all parameters are pregnancy outcomes and should be reported using the mother's participant ID. If an infant is not enrolled in the same study as the mother, any identified birth defects should be reported using the mother's participant ID. However, if an infant is enrolled in the same study as the mother or in another study, any identified birth defects should be reported using the infant's participant ID. Sites should refer to the applicable network standards for reporting abnormal pregnancy outcomes on the CRFs.

DETERMINING SEVERITY GRADE FOR PARAMETERS BETWEEN GRADES

If the severity of an AE could fall in either one of two grades (i.e., the severity of an AE could be either grade 2 or grade 3), sites should select the higher of the two grades.

LABORATORY VALUES

General. An asymptomatic, abnormal laboratory finding without an accompanying AE should not be reported to DAIDS in an expedited timeframe unless it meets protocol-specific reporting requirements. Sites should refer to the applicable network standards for reporting abnormal laboratory findings on the CRFs.

Values below Grade 1. Any laboratory value that is between the ULN and grade 1 (for high values) or the LLN and grade 1 (for low values) should not be graded or reported as an AE. Sites should consult the *Manual for Expedited Reporting of Adverse Events to DAIDS, Version 2.0* and their protocol when making an assessment of the need to report an AE.

Overlap of Local Laboratory Normal Values with Grading Table Ranges. When local laboratory normal values fall within grading table laboratory ranges, the severity grading is based on the ranges in the grading table unless there is a protocol-specific grading criterion for the laboratory value. For example, “Magnesium, Low" has a grade 1 range of 1.2 to < 1.4 mEq/L, while a particular laboratory’s normal range for magnesium may be 1.3 to 2.8 mEq/L. If a study participant’s magnesium laboratory value is 1.3 mEq/L, the laboratory value should be graded as grade 1.

ESTIMATING SEVERITY GRADE FOR PARAMETERS NOT IDENTIFIED IN THE GRADING TABLE

The functional table below should be used to grade the severity of an AE that is not specifically identified in the grading table. In addition, all deaths related to an AE are to be classified as grade 5.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
CARDIOVASCULAR				
Arrhythmia (by ECG or physical examination) *Specify type, if applicable*	No symptoms AND No intervention indicated	No symptoms AND Non-urgent intervention indicated	Non-life-threatening symptoms AND Non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Blood Pressure Abnormalities[4] ***Hypertension*** *(with the lowest reading taken after repeat testing during a visit)* *≥ 18 years of age*	140 to < 160 mmHg systolic OR 90 to < 100 mmHg diastolic	mmHg systolic OR ≥ 100 to < 110 mmHg diastolic≥ 160 to < 180	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
< 18 years of age	> 120/80 mmHg	≥ 95th to < 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	≥ 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
Hypotension	No symptoms	Symptoms corrected with oral fluid replacement	Symptoms AND IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Cardiac Ischemia or Infarction *Report only one*	NA	NA	New symptoms with ischemia (stable angina) OR New testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Heart Failure	No symptoms AND Laboratory or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion	Symptoms at rest or with minimal activity or exertion (e.g., hypoxemia) OR Intervention indicated (e.g., oxygen)	Life-threatening consequences OR Urgent intervention indicated (e.g., vasoactive medications, ventricular assist device, heart transplant)
Hemorrhage (with significant acute blood loss)	NA	Symptoms AND No transfusion indicated	Symptoms AND Transfusion of ≤ 2 units packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children, packed RBCs > 10 cc/kg) indicated

[4] Blood pressure norms for children < 18 years of age can be found in: Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents. *Pediatrics* 2011;128;S213; originally published online November 14, 2011; DOI: 10.1542/peds.20092107C.

Prolonged PR Interval or AV Block *Report only one* *> 16 years of age*	PR interval 0.21 to < 0.25 seconds	PR interval ≥ 0.25 seconds OR Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
≤ 16 years of age	1st degree AV block (PR interval > normal for age and rate)	Type I 2nd degree AV block	block OR Ventricular pause ≥ 3.0 seconds Type II 2nd degree AV	Complete AV block
Prolonged QTc Interval[5]	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Thrombosis or Embolism *Report only one*	NA	Symptoms AND No intervention indicated	Symptoms AND Intervention indicated	Life-threatening embolic event (e.g., pulmonary embolism, thrombus)
DERMATOLOGIC				
Alopecia (scalp only)	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	NA	NA
Bruising	Localized to one area	Localized to more than one area	Generalized	NA
Cellulitis	NA	Non-parenteral treatment indicated (e.g., oral antibiotics, antifungals, antivirals)	IV treatment indicated (e.g., IV antibiotics, antifungals, antivirals)	Life-threatening consequences (e.g., sepsis, tissue necrosis)
Hyperpigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Hypopigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Petechiae	Localized to one area	Localized to more than one area	Generalized	NA

[5] As per Bazett's formula.

Pruritus[6] (without skin lesions)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
Rash *Specify type, if applicable*	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis
ENDOCRINE AND METABOLIC				
Diabetes Mellitus	Controlled without medication	Controlled with medication OR Modification of current medication regimen	Uncontrolled despite treatment modification OR Hospitalization for immediate glucose control indicated	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar nonketotic coma, end organ failure)
Gynecomastia	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing pain with greater than minimal interference with usual social & functional activities	Disfiguring changes AND Symptoms requiring intervention or causing inability to perform usual social & functional activities	NA
Hyperthyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)

[6] For pruritus associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).

Lipoatrophy[7]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA
Lipohypertrophy[8]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA
GASTROINTESTINAL				
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)
Ascites	No symptoms	Symptoms AND Intervention indicated (e.g., diuretics, therapeutic paracentesis)	Symptoms recur or persist despite intervention	Life-threatening consequences
Bloating or Distension *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cholecystitis	NA	Symptoms AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis, perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea *≥ 1 year of age*	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)

[7] Definition: A disorder characterized by fat loss in the face, extremities, and buttocks.
[8] Definition: A disorder characterized by abnormal fat accumulation on the back of the neck, breasts, and abdomen.

< 1 year of age	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (e.g., liquid stools resulting in severe dehydration, hypotensive shock)
Dysphagia or Odynophagia *Report only one and specify location*	Symptoms but able to eat usual diet	Symptoms causing altered dietary intake with no intervention indicated	Symptoms causing severely altered dietary intake with intervention indicated	Life-threatening reduction in oral intake
Gastrointestinal Bleeding	Not requiring intervention other than iron supplement	Endoscopic intervention indicated	Transfusion indicated	Life-threatening consequences (e.g., hypotensive shock)
Mucositis or Stomatitis *Report only one and specify location*	Mucosal erythema	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Life-threatening consequences (e.g., aspiration, choking) OR Tissue necrosis OR Diffuse spontaneous mucosal bleeding
Nausea	Transient (< 24 hours) or intermittent AND No or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptoms with hospitalization not indicated	Symptoms with hospitalization indicated	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)
Perforation (colon or rectum)	NA	NA	Intervention indicated	Life-threatening consequences
Proctitis	Rectal discomfort with no intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Rectal Discharge	Visible discharge	Discharge requiring the use of pads	NA	NA
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
MUSCULOSKELETAL				
Arthralgia	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions

Arthritis	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Myalgia (generalized)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Osteonecrosis	NA	No symptoms but with radiographic findings AND No operative intervention indicated	Bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
Osteopenia[9] ≥ *30 years of age*	BMD t-score -2.5 to -1	NA	NA	NA
< 30 years of age	BMD z-score -2 to -1	NA	NA	NA
Osteoporosis[6] ≥ *30 years of age*	NA	BMD t-score < -2.5	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences
< 30 years of age	NA	BMD z-score < -2	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences
NEUROLOGIC				
Acute CNS Ischemia	NA	NA	Transient ischemic attack	Cerebral vascular accident (e.g., stroke with neurological deficit)
Altered Mental Status (for Dementia, see *Cognitive, Behavioral, or Attentional Disturbance* below)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR Obtundation OR Coma
Ataxia	Symptoms causing no or minimal interference with usual social & functional activities OR No symptoms with ataxia detected on examination	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling symptoms causing inability to perform basic self-care functions

[9] BMD t and z scores can be found in: Kanis JA on behalf of the World Health Organization Scientific Group (2007). Assessment of osteoporosis at the primary health-care level. Technical Report. World Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, UK. 2007: Printed by the University of Sheffield.

Cognitive, Behavioral, or Attentional Disturbance (includes dementia and attention deficit disorder) *Specify type, if applicable*	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part time basis indicated	Disability causing inability to perform usual social & functional activities OR Specialized resources on a fulltime basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated
Developmental Delay *< 18 years of age* *Specify type, if applicable*	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated OR Headache with significant impairment of alertness or other neurologic function
Neuromuscular Weakness (includes myopathy and neuropathy) *Specify type, if applicable*	Minimal muscle weakness causing no or minimal interference with usual social & functional activities OR No symptoms with decreased strength on examination	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory Alteration (includes paresthesia and painful neuropathy) *Specify type, if applicable*	Minimal paresthesia causing no or minimal interference with usual social & functional activities OR No symptoms with sensory alteration on examination	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizures ***New Onset Seizure*** *≥ 18 years of age*	NA	NA	1 to 3 seizures	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)

< 18 years of age (includes new or preexisting febrile seizures)	Seizure lasting < 5 minutes with < 24 hours postictal state	Seizure lasting 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Pre-existing Seizure	NA	Increased frequency from previous level of control without change in seizure character	Change in seizure character either in duration or quality (e.g., severity or focality)	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Syncope	Near syncope without loss of consciousness (e.g., pre-syncope)	Loss of consciousness with no intervention indicated	Loss of consciousness AND Hospitalization or intervention required	NA
PREGNANCY, PUERPERIUM, AND PERINATAL				
Fetal Death or Stillbirth (report using mother's participant ID) *Report only one*	NA	NA	Fetal loss occurring at ≥ 20 weeks gestation	NA
Preterm Delivery[7] (report using mother's participant ID)	Delivery at 34 to < 37 weeks gestational age	Delivery at 28 to < 34 weeks gestational age	Delivery at 24 to < 28 weeks gestational age	Delivery at < 24 weeks gestational age
Spontaneous Abortion or Miscarriage[8] (report using mother's participant ID) *Report only one*	Chemical pregnancy	Uncomplicated spontaneous abortion or miscarriage	Complicated spontaneous abortion or miscarriage	NA
PSYCHIATRIC				

Insomnia	Mild difficulty falling asleep, staying asleep, or waking up early	Moderate difficulty falling asleep, staying asleep, or waking up early	Severe difficulty falling asleep, staying asleep, or waking up early	NA
Psychiatric Disorders (includes anxiety, depression, mania, and psychosis) *Specify disorder*	Symptoms with intervention not indicated OR Behavior causing no or minimal interference with usual social & functional activities	Symptoms with intervention indicated OR Behavior causing greater than minimal interference with usual social & functional activities	Symptoms with hospitalization indicated OR Behavior causing inability to perform usual social & functional activities	Threatens harm to self or others OR Acute psychosis OR Behavior causing inability to perform basic self-care functions
Suicidal Ideation or Attempt *Report only one*	Preoccupied with thoughts of death AND No wish to kill oneself	Preoccupied with thoughts of death AND Wish to kill oneself with no specific plan or intent	Thoughts of killing oneself with partial or complete plans but no attempt to do so OR Hospitalization indicated	Suicide attempted
RESPIRATORY				
Acute Bronchospasm	Forced expiratory volume in 1 second or peak flow reduced to ≥ 70 to < 80% OR Mild symptoms with intervention not indicated	Forced expiratory volume in 1 second or peak flow 50 to < 70% OR Symptoms with intervention indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Forced expiratory volume in 1 second or peak flow 25 to < 50% OR Symptoms causing inability to perform usual social & functional activities	Forced expiratory volume in 1 second or peak flow < 25% OR Life-threatening respiratory or hemodynamic compromise OR Intubation
Dyspnea or Respiratory Distress *Report only one*	Dyspnea on exertion with no or minimal interference with usual social & functional activities OR Wheezing OR Minimal increase in respiratory rate for age	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities OR Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 to < 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilator support indicated (e.g., CPAP, BPAP, intubation)
SENSORY				
Hearing Loss ≥ *12 years of age*	NA	Hearing aid or intervention not indicated	Hearing aid or intervention indicated	Profound bilateral hearing loss (> 80 dB at 2 kHz and above) OR Non-serviceable hearing (i.e., >50 dB audiogram and <50% speech discrimination)

< 12 years of age (based on a 1, 2, 3, 4, 6 and 8 kHz audiogram)	> 20 dB hearing loss at ≤ 4 kHz	> 20 dB hearing loss at > 4 kHz	> 20 dB hearing loss at ≥ 3 kHz in one ear with additional speech language related services indicated (where available) OR Hearing loss sufficient to indicate therapeutic intervention, including hearing aids	Audiologic indication for cochlear implant and additional speech language related services indicated (where available)
Tinnitus	Symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Symptoms causing inability to perform usual social & functional activities	NA
Uveitis	No symptoms AND Detectable on examination	Anterior uveitis with symptoms OR Medicamylasal intervention indicated	Posterior or panuveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions
Visual Changes (assessed from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)
SYSTEMIC				
Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA

Cytokine Release Syndrome[10]	Mild signs and symptoms AND Therapy (i.e., antibody infusion) interruption not indicated	Therapy (i.e., antibody infusion) interruption Responds promptly to symptomatic treatment OR Prophylactic medications indicated for ≤ 24 indicated AND hours	Prolonged severe signs and symptoms OR Recurrence of symptoms following initial improvement	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Fatigue or Malaise *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating symptoms of fatigue or malaise causing inability to perform basic self-care functions
Fever (non-axillary temperatures only)	38.0 to < 38.6°C or 100.4 to < 101.5°F	≥ 38.6 to < 39.3°C or ≥ 101.5 to < 102.7°F	≥ 39.3 to < 40.0°C or ≥ 102.7 to < 104.0°F	≥ 40.0°C or ≥ 104.0°F
Pain[11] (not associated with study agent injections and not specified elsewhere) *Specify location*	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization indicated
Serum Sickness[12]	Mild signs and symptoms	Moderate signs and symptoms AND Intervention indicated (e.g., antihistamines)	Severe signs and symptoms AND Higher level intervention indicated (e.g., steroids or IV fluids)	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Systemic **Underweight**[13] *> 5 to 19 years of age*	NA	WHO BMI z-score < -2 to ≤ -3	WHO BMI z-score < -3	WHO BMI z-score < -3 with life-threatening consequences
2 to 5 years of age	NA	WHO Weight-for-height z-score < -2 to ≤ -3	WHO Weight-for-height z-score < -3	WHO Weight-for-height z-score < -3 with life-threatening consequences
< 2 years of age	NA	WHO Weight-for-length z-score < -2 to ≤ -3	WHO Weight-for-length z-score < -3	WHO Weight-for-length z-score < -3 with life-threatening consequences
Weight Loss (excludes postpartum weight loss)	NA	5 to < 9% loss in body weight from baseline	≥ 9 to < 20% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated (e.g., tube

[10] Definition: A disorder characterized by nausea, headache, tachycardia, hypotension, rash, and/or shortness of breath.
[11] For pain associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).
[12] Definition: A disorder characterized by fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.
[13] WHO reference tables may be accessed by clicking the desired age range or by accessing the following URLs:
http://www.who.int/growthref/who2007_bmi_for_age/en/ for participants > 5 to 19 years of age and
http://www.who.int/childgrowth/standards/chart_catalogue/en/ for those ≤ 5 years of age.

				feeding, total parenteral nutrition)
URINARY				
Urinary Tract Obstruction	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences
SITE REACTIONS TO INJECTIONS AND INFUSIONS				
Injection Site Pain or Tenderness *Report only one*	Pain or tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain or tenderness causing inability to perform usual social & functional activities	Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness[14] *Report only one* *> 15 years of age*	2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities	≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities	≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one* *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*
≤ 15 years of age	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*

[14] Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area.

Injection Site Pruritus	Itching localized to the injection site that is relieved spontaneously or in < 48 hours of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA
LABORATORY VALUES				
CHEMISTRIES				
Acidosis	NA	pH ≥ 7.3 to < LLN	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, Low (g/dL; *g/L*)	3.0 to < LLN *30 to < LLN*	≥ 2.0 to < 3.0 *≥ 20 to < 30*	< 2.0 *< 20*	NA
Alkaline Phosphatase, High	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Alkalosis	NA	pH > ULN to ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT or SGPT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Amylase (Pancreatic) or Amylase (Total), High *Report only one*	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
AST or SGOT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Bicarbonate, Low (mEq/L; *mmol/L*)	16.0 to < LLN *16.0 to < LLN*	11.0 to < 16.0 *11.0 to < 16.0*	8.0 to < 11.0 *8.0 to < 11.0*	< 8.0 *< 8.0*
Bilirubin ***Direct Bilirubin[15], High*** *> 28 days of age*	NA	NA	> ULN	> ULN with life-threatening consequences (e.g., signs and symptoms of liver failure)
≤ 28 days of age	ULN to ≤ 1 mg/dL	> 1 to ≤ 1.5 mg/dL	> 1.5 to ≤ 2 mg/dL	> 2 mg/dL
Total Bilirubin, High *> 28 days of age*	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN
≤ 28 days of age	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates
Calcium, High (mg/dL; *mmol/L*)	10.6 to < 11.5 *2.65 to < 2.88*	11.5 to < 12.5 *2.88 to < 3.13*	12.5 to < 13.5 *3.13 to < 3.38*	

[15] Direct bilirubin > 1.5 mg/dL in a participant < 28 days of age should be graded as grade 2, if < 10% of the total bilirubin.

≥ 7 days of age				≥ 13.5 *≥ 3.38*
< 7 days of age	11.5 to < 12.4 *2.88 to < 3.10*	12.4 to < 12.9 *3.10 to < 3.23*	12.9 to < 13.5 *3.23 to < 3.38*	≥ 13.5 *≥ 3.38*
Calcium (Ionized), High (mg/dL; *mmol/L*)	> ULN to < 6.0 *> ULN to < 1.5*	6.0 to < 6.4 *1.5 to < 1.6*	6.4 to < 7.2 *1.6 to < 1.8*	≥ 7.2 *≥ 1.8*
Calcium, Low (mg/dL; *mmol/L*)				
≥ 7 days of age	7.8 to < 8.4 *1.95 to < 2.10*	7.0 to < 7.8 *1.75 to < 1.95*	6.1 to < 7.0 *1.53 to < 1.75*	< 6.1 < *1.53*
< 7 days of age	6.5 to < 7.5 *1.63 to < 1.88*	6.0 to < 6.5 *1.50 to < 1.63*	5.50 to < 6.0 *1.38 to < 1.50*	< 5.50 *< 1.38*
Calcium (Ionized), Low (mg/dL; *mmol/L*)	< LLN to 4.0 *< LLN to 1.0*	3.6 to < 4.0 *0.9 to < 1.0*	3.2 to < 3.6 *0.8 to < 0.9*	< 3.2 *< 0.8*
Cardiac Troponin I, High	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the local laboratory
Creatine Kinase, High	3 to < 6 x ULN	6 to < 10 x ULN	10 to < 20 x ULN	≥ 20 x ULN
Creatinine, High	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase of > 0.3 mg/dL above baseline	> 1.8 to < 3.5 x ULN OR Increase of 1.5 to < 2.0 x above baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x above baseline
Creatinine Clearance[16] or eGFR, Low *Report only one*	NA	< 90 to 60 ml/min or ml/min/1.73 m^2 OR 10 to < 30% decrease from baseline	< 60 to 30 ml/min or ml/min/1.73 m^2 OR ≥ 30 to < 50% decrease from baseline	< 30 ml/min or ml/min/1.73 m^2 OR ≥ 50% decrease from baseline or dialysis needed
Glucose (mg/dL; *mmol/L*)				
Fasting, High	110 to 125 *6.11 to < 6.95*	> 125 to 250 *6.95 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	> 500 *≥ 27.75*
Nonfasting, High	116 to 160 *6.44 to < 8.89*	> 160 to 250 *8.89 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	> 500 *≥ 27.75*
Glucose, Low (mg/dL; *mmol/L*)				
≥ 1 month of age	55 to 64 *3.05 to 3.55*	40 to < 55 *2.22 to < 3.05*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
< 1 month of age	50 to 54 *2.78 to 3.00*	40 to < 50 *2.22 to < 2.78*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
Lactate, High	ULN to < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences
Lipase, High	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN

[16] Use the applicable formula (i.e., Cockroft-Gault in mL/min or Schwatrz in mL/min/1.73m^2).

Lipid Disorders (mg/dL; *mmol/L*) ***Cholesterol, Fasting, High*** *≥ 18 years of age*	200 to < 240 *5.18 to < 6.19*	240 to < 300 *6.19 to < 7.77*	≥ 300 *≥ 7.77*	NA
< 18 years of age	170 to < 200 *4.40 to < 5.15*	200 to < 300 *5.15 to < 7.77*	≥ 300 *≥ 7.77*	NA
LDL, Fasting, High ≥ *18 years of age*	130 to < 160 *3.37 to < 4.12*	160 to < 190 *4.12 to < 4.90*	≥ 190 *≥ 4.90*	NA
> 2 to < 18 years of age	110 to < 130 *2.85 to < 3.34*	130 to < 190 *3.34 to < 4.90*	≥ 190 ≥ *4.90*	NA
Triglycerides, Fasting, High	150 to 300 *1.71 to 3.42*	>300 to 500 *>3.42 to 5.7*	>500 to < 1,000 *>5.7 to 11.4*	> 1,000 *> 11.4*
Magnesium[17], Low (mEq/L; *mmol/L*)	1.2 to < 1.4 *0.60 to < 0.70*	0.9 to < 1.2 *0.45 to < 0.60*	0.6 to < 0.9 *0.30 to < 0.45*	< 0.6 *< 0.30*
Phosphate, Low (mg/dL; *mmol/L*) *> 14 years of age*	2.0 to < LLN *0.81 to < LLN*	1.4 to < 2.0 *0.65 to < 0.81*	1.0 to < 1.4 *0.32 to < 0.65*	< 1.0 *< 0.32*
1 to 14 years of age	3.0 to < 3.5 *0.97 to < 1.13*	2.5 to < 3.0 *0.81 to < 0.97*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
< 1 year of age	3.5 to < 4.5 *1.13 to < 1.45*	2.5 to < 3.5 *0.81 to < 1.13*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
Potassium, High (mEq/L; *mmol/L*)	5.6 to < 6.0 *5.6 to < 6.0*	6.0 to < 6.5 *6.0 to < 6.5*	6.5 to < 7.0 *6.5 to < 7.0*	≥ 7.0 *≥ 7.0*
Potassium, Low (mEq/L; *mmol/L*)	3.0 to < 3.4 *3.0 to < 3.4*	2.5 to < 3.0 *2.5 to < 3.0*	2.0 to < 2.5 *2.0 to < 2.5*	< 2.0 *< 2.0*
Sodium, High (mEq/L; *mmol/L*)	146 to < 150 *146 to < 150*	150 to < 154 *150 to < 154*	154 to < 160 *154 to < 160*	≥ 160 *≥ 160*
Sodium, Low (mEq/L; *mmol/L*)	130 to < 135 *130 to < 135*	125 to < 130 *125 to < 135*	121 to < 125 *121 to < 125*	≤ 120 *≤ 120*
Uric Acid, High (mg/dL; *mmol/L*)	7.5 to < 10.0 *0.45 to < 0.59*	10.0 to < 12.0 *0.59 to < 0.71*	12.0 to < 15.0 *0.71 to < 0.89*	≥ 15.0 ≥ *0.89*
HEMATOLOGY				
Absolute CD4+ Count, Low (cell/mm^3; *cells/L*) *> 5 years of age (not HIV infected)*	300 to < 400 *300 to < 400*	200 to < 300 *200 to < 300*	100 to < 200 *100 to < 200*	< 100 *< 100*

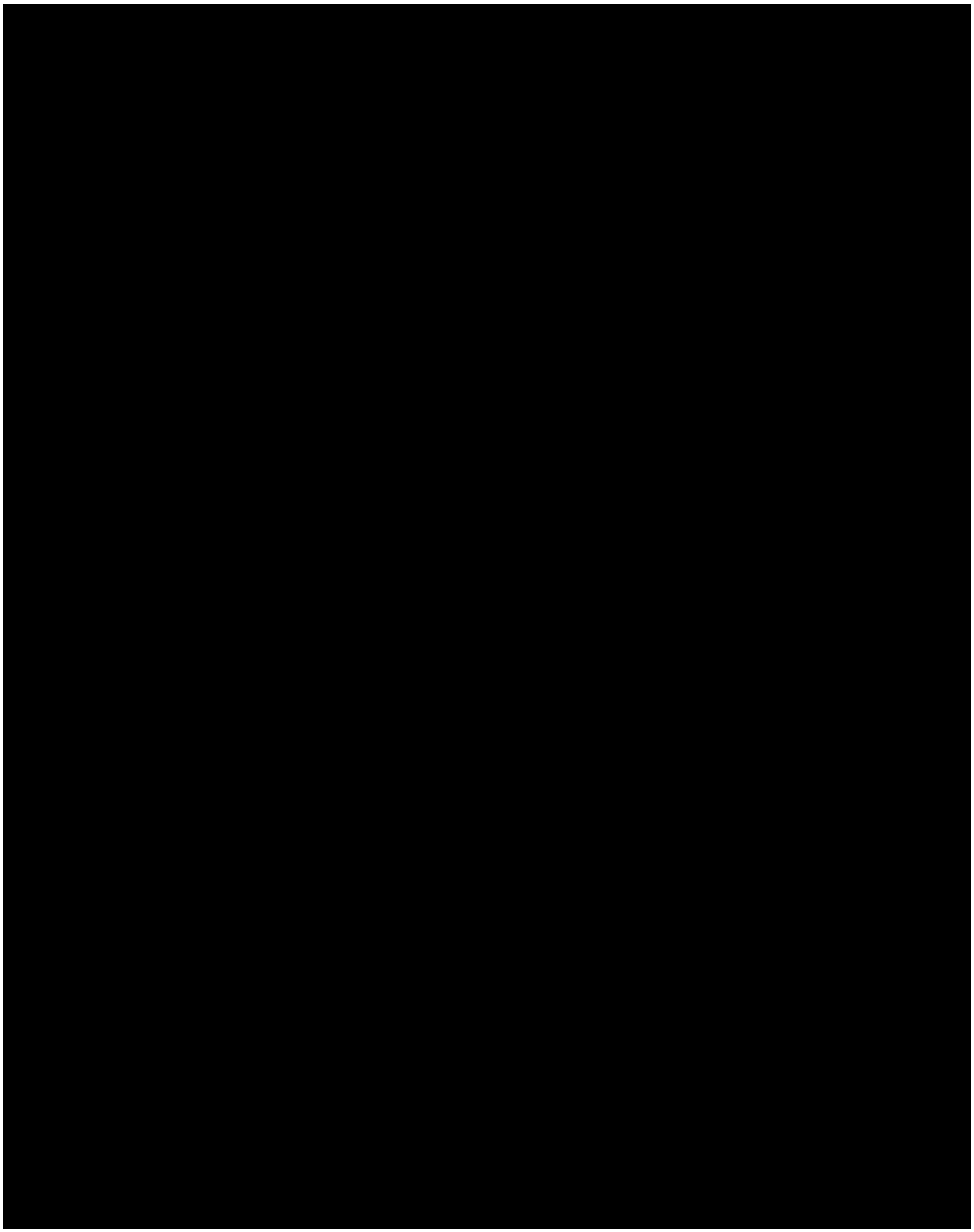
[17] To convert a magnesium value from mg/dL to mmol/L, laboratories should multiply by 0.4114.

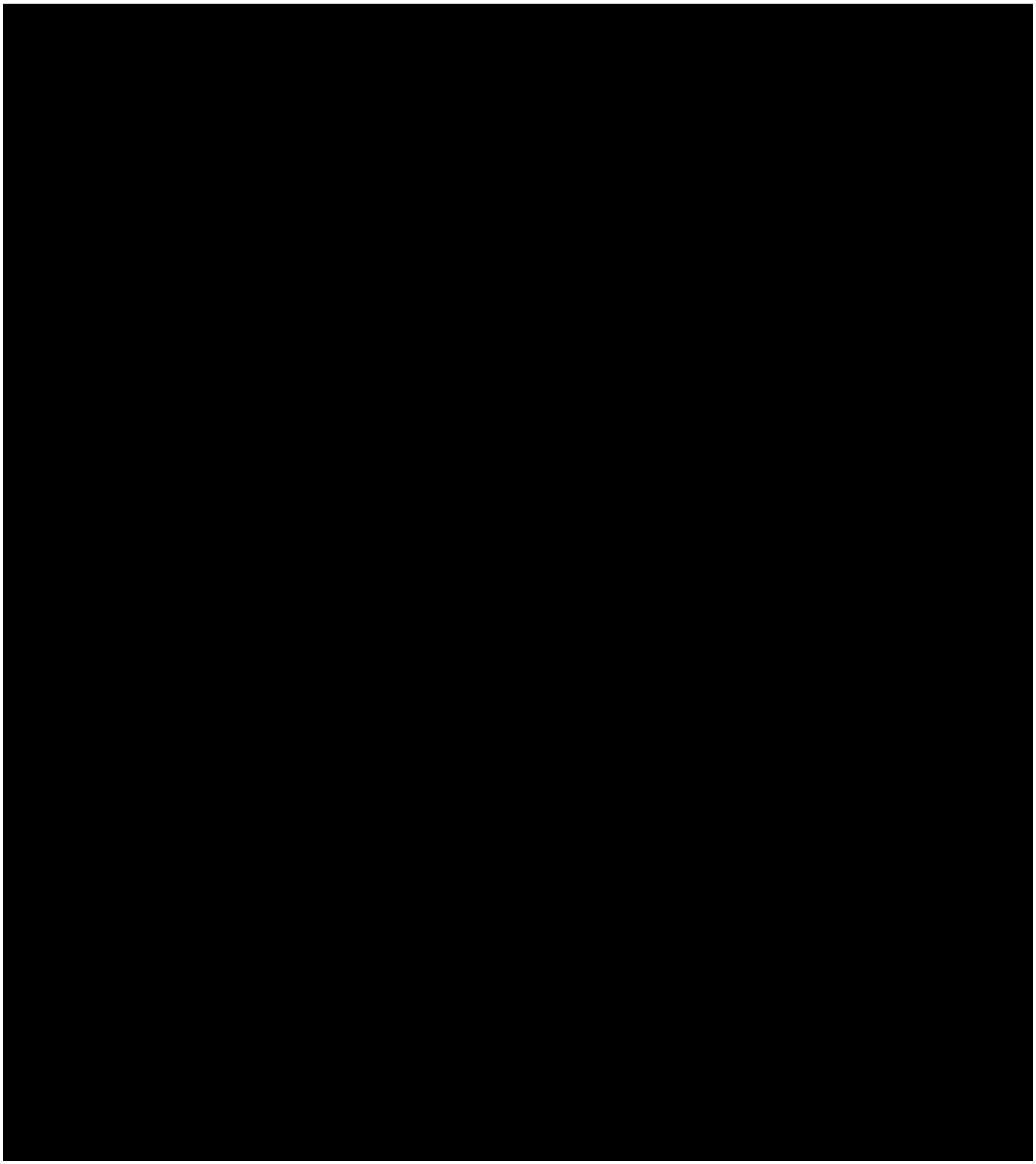
Absolute Lymphocyte Count, Low (cell/mm^3; *cells/L*) *> 5 years of age (not HIV infected)*	600 to < 650 *0.600 x 10^9 to < 0.650 x 10^9*	500 to < 600 *0.500 x 10^9 to < 0.600 x 10^9*	350 to < 500 *0.350 x 10^9 to < 0.500 x 10^9*	< 350 *< 0.350 x 10^9*
Absolute Neutrophil Count (ANC), Low (cells/mm^3; *cells/L*) *> 7 days of age*	800 to 1,000 *0.800 x 10^9 to 1.000 x 10^9*	600 to 799 *0.600 x 10^9 to 0.799 x 10^9*	400 to 599 *0.400 x 10^9 to 0.599 x 10^9*	< 400 *< 0.400 x 10^9*
2 to 7 days of age	1,250 to 1,500 *1.250 x 10^9 to 1.500 x 10^9*	1,000 to 1,249 *1.000 x 10^9 to 1.249 x 10^9*	750 to 999 *0.750 x 10^9 to 0.999 x 10^9*	< 750 *< 0.750 x 10^9*
≤ 1 day of age	4,000 to 5,000 *4.000 x 10^9 to 5.000 x 10^9*	3,000 to 3,999 *3.000 x 10^9 to 3.999 x 10^9*	1,500 to 2,999 *1.500 x 10^9 to 2.999 x 10^9*	< 1,500 *< 1.500 x 10^9*
Fibrinogen, Decreased (mg/dL; *g/L*)	100 to < 200 *1.00 to < 2.00* OR 0.75 to < 1.00 x LLN	75 to < 100 *0.75 to < 1.00* OR ≥ 0.50 to < 0.75 x LLN	50 to < 75 *0.50 to < 0.75* OR 0.25 to < 0.50 x LLN	< 50 *< 0.50* OR < 0.25 x LLN OR Associated with gross bleeding
Hemoglobin[18], Low (g/dL; *mmol/L*)[19] *≥ 13 years of age (male only)*	10.0 to 10.9 *6.19 to 6.76*	9.0 to < 10.0 *5.57 to < 6.19*	7.0 to < 9.0 *4.34 to < 5.57*	< 7.0 *< 4.34*
≥ 13 years of age (female only)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*
57 days of age to < 13 years of age (male and female)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*
36 to 56 days of age (male and female)	8.5 to 9.6 *5.26 to 5.99*	7.0 to < 8.5 *4.32 to < 5.26*	6.0 to < 7.0 *3.72 to < 4.32*	< 6.0 *< 3.72*
22 to 35 days of age (male and female)	9.5 to 11.0 *5.88 to 6.86*	8.0 to < 9.5 *4.94 to < 5.88*	6.7 to < 8.0 *4.15 to < 4.94*	< 6.7 *< 4.15*
8 to ≤ 21 days of age (male and female)	11.0 to 13.0 *6.81 to 8.10*	9.0 to < 11.0 *5.57 to < 6.81*	8.0 to < 9.0 *4.96 to < 5.57*	< 8.0 < *4.96*
≤ 7 days of age (male and female)	13.0 to 14.0 *8.05 to 8.72*	10.0 to < 13.0 *6.19 to < 8.05*	9.0 to < 10.0 *5.59 to < 6.19*	< 9.0 *< 5.59*
INR, High	1.1 to < 1.5 x ULN	1.5 to < 2.0 x ULN	2.0 to < 3.0 x ULN	≥ 3.0 x ULN

[18] Male and female sex are defined as sex at birth.

[19] The conversion factor used to convert g/dL to mmol/L is 0.6206 and is the most commonly used conversion factor. For grading hemoglobin results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using the appropriate conversion factor for the particular laboratory.

(not on anticoagulation therapy)				
Methemoglobin (% hemoglobin)	5.0 to < 10.0%	10.0 to < 15.0%	15.0 to < 20.0%	≥ 20.0%
PTT, High (not on anticoagulation therapy)	1.1 to < 1.66 x ULN	1.66 to < 2.33 x ULN	2.33 to < 3.00 x ULN	≥ 3.00 x ULN
Platelets, Decreased (cells/mm^3; *cells/L*)	100,000 to < 124,999 *100.000 x 10^9 to < 124.999 x 10^9*	50,000 to < 100,000 *50.000 x 10^9 to < 100.000 x 10^9*	25,000 to < 50,000 *25.000 x 10^9 to < 50.000 x 10^9*	< 25,000 *< 25.000 x 10^9*
PT, High (not on anticoagulation therapy	1.1 to < 1.25 x ULN	1.25 to < 1.50 x ULN	1.50 to < 3.00 x ULN	≥ 3.00 x ULN
WBC, Decreased (cells/mm^3; *cells/L*)				
> 7 days of age	2,000 to 2,499 *2.000 x 10^9 to 2.499 x 10^9*	1,500 to 1,999 *1.500 x 10^9 to 1.999 x 10^9*	1,000 to 1,499 *1.000 x 10^9 to 1.499 x 10^9*	< 1,000 *< 1.000 x 10^9*
≤ 7 days of age	5,500 to 6,999 *5.500 x 10^9 to 6.999 x 10^9*	4,000 to 5,499 *4.000 x 10^9 to 5.499 x 10^9*	2,500 to 3,999 *2.500 x 10^9 to 3.999 x 10^9*	< 2,500 *< 2.500 x 10^9*
URINALYSIS				
Glycosuria (random collection tested by dipstick)	Trace to 1+ or ≤ 250 mg	2+ or > 250 to ≤ 500 mg	> 2+ or > 500 mg	NA
Hematuria (not to be reported based on dipstick findings or on blood believed to be of menstrual origin)	6 to < 10 RBCs per high power field	≥ 10 RBCs per high power field	Gross, with or without clots OR With RBC casts OR Intervention indicated	Life-threatening consequences
Proteinuria (random collection tested by dipstick)	1+	2+	3+ or higher	NA





PROTOCOL AMENDMENT 1: OTL-101-4 (18 JANUARY 2017)

EFFICACY AND SAFETY OF A CRYOPRESERVED FORMULATION OF AUTOLOGOUS CD34+ HEMATOPOIETIC STEM CELLS TRANSDUCED EX VIVO WITH EFS LENTIVIRAL VECTOR ENCODING FOR HUMAN ADA GENE IN SUBJECTS WITH SEVERE COMBINED IMMUNODEFICIENCY DUE TO ADENOSINE DEAMINASE DEFICIENCY

STUDY PROTOCOL
STUDY number: OTL-101-4
PRODUCT: A CRYOPRESERVED FORMULATION OF AUTOLOGOUS CD34+ HEMATOPOIETIC STEM CELLS TRANSDUCED EX VIVO WITH EFS LENTIVIRAL VECTOR ENCODING FOR THE HUMAN ADA GENE (OTL-101)
[IND number: 15440]

Original Protocol, Version 1.0: 24 October 2016
Protocol Amendment 1, Version 2.0: 18 January 2017

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

STUDY number: OTL-101-4
Protocol Amendment 1, Version 2.0: 18 January 2017

RATIONALE AND SUMMARY OF CHANGES

The purpose of protocol amendment 1 (18 January 2017) is to provide further clarity on procedures, align consistent language and terms throughout the document, and provide flexibility for blood sample volume due to the age of the subjects to be treated under the protocol.

This document will summarise the key changes accordingly:

1. Baseline immune function tests can be performed following consent/assent and prior to conditioning for subjects (particularly infants) where blood volume is restricted per visit. The window for screening assessments has also been aligned with standard practice to permit prior assessments to be used within 60 days to confirm eligibility.
2. A vector integration analysis has been added to assessments post-transplant.
3. The protocol has been updated in terms of current available information on the disease, current treatment options, trial details for the OTL-101 program, and permitted concomitant medication (including risks and benefits).
4. Language consistency applied throughout the document:
    - Age of subjects eligible for inclusion,
    - Quality of life tools to be used (with an added statement to apply age appropriate tools),
    - Hematopoietic instead of immune reconstitution,
    - Removal of “possible RT-PCR” from gene transduction assessment
    - Duplicate text removed.
5. Further details are provided regarding the bone marrow harvesting procedures and the requirement to obtain a back-up sample at bone marrow harvest. Further clarification on when to use the back-up sample and the withdrawal of subjects from the study if there is no evidence of hematopoietic reconstitution.
6. Busulfan pharmacokinetic data description is removed from the second analysis.
7. Further clarification of OTL-101 product storage and administration. Dose calculation forms (single and multi-bag) have been appended to the protocol for ease of use by the Investigator. In addition, an accountability log is provided in the appendix to ensure adequate recording and verification of OTL-101 product use.
8. Clarification on a post-registry study that subjects will be invited to participate in.
9. Subjects that are enrolled but do not receive the OTL-101 product (regardless of reason) will be included in the intention-to-treat population.
10. Administrative changes and typographical errors (which are not shown in the table below).

PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale. Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Revision	Relates to Change No.
Schedule of assessments: Table 1	Assessment timeframes have been specified in the table for Screening, pre-harvest assessment, bone marrow harvest, and baseline visits for clarity. Text added to parameter assessment in the table: ADA-SCID diagnosis confirmation Footnote added: 11. The immune function tests required for Visit 3, Assessment C can be performed at any point after provision of consent/assent and prior to busulfan administration in younger subjects if deemed appropriate by the Investigator. 12. These test can be performed at anytime following BM harvest (Visit 2: Assessment B) and prior to OTL-101 administration (Visit 3: Assessment F) in younger subjects if deemed appropriate by the Investigator.	4 1
Schedule of assessments: Table 2 5.1.7 Follow-Up (Visits 4 to 11)	Vector integration analysis added to the table at Month 3, Month 6, Month 12, Month 18, and Month 24 visits. Antibodies to tetanus toxoid assessment removed at Month 6 visit. FACS T-cells/myeloid by PCR for ~~RCL~~VCN	2 4
1.1.2 Treatment Options	Two ~~current~~ therapeutic methods are currently used for the treatment of ADA SCID, hematopoietic stem cell transplantation (HSCT) and ADA enzyme replacement therapy (ERT) but both options have significant limitations.	3
1.1.2 Treatment Options	Subheadings added: 1.1.2.1 Hematopoietic Stem Cell Transplantation 1.1.2.2 Gene Therapy	3
1.1.2.2 Gene	Paragraph added:	3

Section No./Title	Revision	Relates to Change No.
Therapy	No gene therapy treatment has been approved in United States (US) to date. In Europe, Strimvelis® is approved for the "treatment of subjects with ADA-SCID, for whom no suitable HLA matched related stem cell donor is available". Strimvelis® is a GRV based ex vivo gene therapy treatment.	
1.4.1 Potential Benefits	Sentence added to third paragraph: Durable, life-long clinical benefit is highly dependent on the successful engraftment of genetically modified long term repopulating HSC. The improved correction of HSCs should result in improved immune recovery due to correction of both T and B cell populations. Edits to sixth paragraph, third sentence: The longest follow-up is ~~4 years~~58 months.	3
1.4.2.1 Potential Risks Related to the Protocol Treatment	Bullet 1, third sentence: In current LV ~~studies~~subjects (39 subjects treated to date), there have been no cases of oncogenicity.	3
1.4.2.3 Potential Risks Related to Concomitant Therapy	Busulfan Busulfan is used for conditioning prior to the re-infusion of the HSCs. The busulfan dose to be used in this clinical study will target a specific area under the curve (AUC), which will ensure more precise dosing and may reduce the risks of toxicity. The risks attached to busulfan are minimal, although there is a very small possibility of busulfan-related cancer. More common risks associated with busulfan at higher doses include nausea, vomiting, diarrhoea, constipation, loss of appetite, mouth sores, stomach/abdominal pain, dizziness, swelling ankles/feet/hand, flushing, headache, ~~or~~ trouble sleeping and a period of neutropaenia. However, the above systemic side-effects have not been seen at the proposed busulfan dose for this study. In the Sponsor's experience to date, there have been no busulfan-related serious adverse events (SAEs). Pegylated-ADA	3

Section No./Title	Revision	Relates to Change No.
	The withdrawal of PEG-ADA, which is required 1 month after OTL-101 administration, can have adverse effects on immunity, as well as on other organ systems. It is possible that a very severe infection may develop during this time, which could be fatal if the subject does not respond to OTL-101 infusion. In the Sponsor's experience to date, there have been no ~~busulfan-related serious adverse events (SAEs), neither~~ severe infections nor causalities, related to stopping PEG-ADA ERT, 1 month after OTL-101 infusion as per protocol.	
2.1 Purpose of the Study	Eigth sentence: Three subjects <u>have already been treated with cryopreserved cells (with a different cryoprotectant as the one used under this protocol); two patients treated under Compassionate Use Program and one patient treated as an exemption in the US</u>~~from the Phase I/II studies conducted at GOSH/UCL and UCLA have received a cryopreserved formulation (not the exact cyropreseved formulation used in this protocol)~~.	3
2.2.3 Exploratory Study Objectives 9.3.4 Exploratory Efficacy	<u>Vector integration analysis</u> added to the list of exploratory parameters.	2
3.1 General Design and Study Scheme	First paragraph: This is a prospective, non-randomized, single-cohort, longitudinal, single-center, clinical study designed to assess the efficacy and safety of OTL-101 cryopreserved formulation administered in ADA-SCID subjects aged between <u>≥30 days to <18</u> ~~30 days and 17~~ years of age, who are not eligible for an HLA-matched sibling/family donor and meeting the inclusion/exclusion criteria. <u>This study aims to recruit 10 evaluable subjects.</u> Fourth paragraph: Eligible subjects will be hospitalized to undergo the harvesting of autologous CD34+ cells. <u>A backup harvest of non-transduced CD34+cells will be obtained during the harvesting procedure to be used in the event of i) product damage during the thawing of the GTMP that would</u>	4 5

Section No./Title	Revision	Relates to Change No.
	prevent infusion of the GTMP although the patient has already been conditioned or ii) lack of engraftment/hematopoeitic reconstitution 42 days post infusion of the protocol treatment. A failure of hematologic reconstitution is defined as at least two of the following: absolute neutophil count (ANC) <200/mm^3, platelets <20,000/mm^3 without transfusions, hemaglobin (Hb) <8.0 g/dl without transfusions on three independent and consecutive determinations over at least 10 days beyond Day 42 from initial GTMP infusion. Sixth paragraph: For subjects who have successfully received the OTL-101 product, PEG-ADA ERT will be discontinued at Day+30 (+/-3) after the transplant. After their discharge from hospital, the subjects will be seen at regular intervals to review their history, perform examinations and draw blood samples at Months 1, 3, 6, 9, 12, 18, and 24. Any medically-indicated interventions will be determined at these visits. Hematopoietic reconstitution will be assessed at Day 42, and in the event of no reconstitution the backup HSC sample will be administered. Hematapoietic reconstitution will be reassessed at Month 6 visit and if there is still no evidence of cellular recovery, the subject will be given ERT and/or HSCT, if available, and deemed appropriate by the Investigator. After Month 24 visit, the subjects will have completed the study and may enter a long term registry.	5
3.1 General Design and Study Scheme 9.6.5 Efficacy Analyses	Busulfan pharmacokinetic (PK) data will not be summarised as part of the second analysis. This will only be performed as part of the third analysis.	6
3.1 General Design and Study Scheme	Figure 1 has been revised to reflect the above changes. Figure footnotes and abbreviations added: # Backup sample will be taken in the event of product damage or failure to achieve hematopoietic reconstitution. * The timeframe for the cryopreservation process may vary Abbreviations: ADA= Adenosine deaminase, AUC = area under the curve, BM = bone marrowm, EFS = Elongation Factor 1α	5

Section No./Title	Revision	Relates to Change No.
	Short form, ERT = enzyme replacement therapy, LV = lentivirus vector.	
3.2.1 Primary Efficacy Evaluations and Endpoints	Bullet 1, last sentence: This data will be used to compare subject data from the ongoing Phase I/II ~~ongoing studies~~ study using the fresh formulation.	4
3.4.1 Protocol Treatment	The protocol treatment, OTL-101, is manufactured under Good Manufacturing Practice (GMP) guidelines, using the subject's own $CD34^+$ HSCs. OTL-101 is formulated in Cryostor CS5, filled into cryobags, cryopreserved and then stored in the vapor phase of liquid nitrogen (LN_2). Final product dose and infusion volume calculations are described in Section 6.1.1 and Section 17: Appendix 1. On the day the subject is to be infused, the cryopreserved OTL-101 product bag(s) will be transported to the bedside in a dry nitrogen shipping container. Immediately prior to infusion, the bag(s) will be removed from the shipping container and thawed using either a plasma thawing device or a pre-warmed ~~sterile~~ water bath (using sterile water). For thawing, the OTL-101 product bag(s) will be placed in an outer bag sleeve in case of a leak. After thawing, the OTL-101 product bag(s) will be attached to a sterile intravenous line fitted with a three-way stopcock and sterile syringe. The cells will be pulled into the syringe from the bag(s), the stopcock will be turned and then the contents of the syringe will be pushed through the intravenous line into the recipient. The OTL-101 product will be labeled as the Study Agent. Please note: when more than one bag is required to be infused, only one OTL-101 product bag per hour will be administered (Section 6.1.1).	7
3.5 Duration of Subject Participation	Sentence added at the end of this section: After finalization of this study subjects will be invited and encouraged to participate in a registry for long term data collections according to guideline recommendations.	8
3.6.1 Subject Stopping Rules and Criteria for Discontinuation	Bullet 2: If two subjects experience prolonged unresponsive pancytopenia, defined as an initial failure of hematologic reconstitution which does not improve following the administration of the subject's autologous back-up cells. A failure of hematologic reconstitution is defined as at least two of the following: absolute neutophil count (ANC) $<200/mm^3$, platelets $<20,000/mm^3$ without	5

Section No./Title	Revision	Relates to Change No.
	transfusions, hemaglobin (Hb) <8.0 g/dl without transfusions on three independent and consecutive determinations ~~over at least 10 days beyond Day 42 from initial GTMP infusion~~. ~~If these conditions are met, the subject's autologous back-up cells will be infused via the intravenous route.~~ If, at Day 90 following the initial infusion of OTL-101, the failure of hematologic reconstitution persists after the subject's autologous back-up cells have been infused, the subject can be said to be experiencing prolonged unresponsive pancytopenia. The subject should then receive standard care for pancytopenia.	
3.6.1 Subject Stopping Rules and Criteria for Discontinuation	Paragraph following the list stopping criteria: Any such finding will be discussed with the Sponsor and DSMB, and an agreement should be made and documented to detail the conditions under which the study can be resumed before enrolling the next subject~~which must agree to the conditions under which the study can be resumed before enrolling the next subject~~. Evaluations of the study endpoints of subjects already enrolled and who have received OTL-101, will continue	4
3.7.1 OTL-101 Storage and Security	Sentence added: A backup harvest of non-transduced CD34+ cells will be retained in i) the event of product damage during the thawing of the GTMP that would prevent infusion of the GTMP although the patient has already been conditioned or ii) lack of engraftment/hematopoeitic reconstitution at 42 days post GTMP (see Section 3.4).	5
3.7.2 OTL-101 Production	Bullet 1: **NOTE:** On Day 1, a back-up fraction of mononuclear cells will be cryopreserved . This back-up will constitute ~~3.0~~5.0 x 10e7 mononuclear cells/kg OR 3.0 x 10e7 total nucleated cells/kg.	5
3.7.3 OTL-101 Administration	Sentence added to the end of the first paragraph: The OTL-101 product will be labeled as the Study Agent. Sentence added to the end of the second paragraph: The OTL-101 product should be stored in the vapor phase of liquid nitrogen (LN_2) until required to be infused into the subject (see Section 3.4.1).	5

Section No./Title	Revision	Relates to Change No.
	Text added to second bullet: (including the certificate of analysis [CofA]) Edits to the third paragraph: Final product dose and infusion volume calculations are specified in Section 6.1.1. The cryobag(s) will then be thawed using either a plasma thawing device or a pre-warmed ~~sterile~~ water bath (using sterile water). For thawing, the OTL-101 product bag will be placed in an outer bag sleeve in case of any fracture. Sentence addd to the end of the fourth paragraph: Please note: when more than one bag is required to be infused, only one OTL-101 product bag per hour will be administered (Section 6.1.1).	
3.7.4 OTL-101 Accountability	Text added after the first sentence: A specific form for the recording of the product traceability can be found in Section 17, Appendix 1 (Global Investigational Product Accountability Log).	5
5.1 Study Visits	Consistency applied when referencing the quality of life assessments performed at Screening, Month 6, Month 12, Month 18, Month 24 and early termination visits: Evaluation of quality of life, as measured by the Karnofsky/Lansky scale and questions relevant to generall well-being, ~~absences from~~ school attendance, and ability to practice sports, respectively~~a quality of life questionnaire and cognitive development~~	4
5.1.1 Screening (Visit 1)	First paragraph, text edited: Written informed consent must be obtained prior to any screening procedures (unless routine medical care assessments are being used within 60 days of the harvest visit as screening assessments). A signed and dated informed consent form will be obtained from the parent/legal guardian (according to local law requirements), and a signed and dated assent form will be obtained from each subject (aged ≥7 years) ~~before screening procedures~~, if required according to local regulations. Evaluations obtained as part of routine medical care and performed during the screening period may be used in place of the study specific evaluations if performed within 60 days of the harvest visit (Visit 2: Assessment B). The parents/legal guardians of each subject	1

Section No./Title	Revision	Relates to Change No.
	will acknowledge and agree to the possible use of this information for the study by giving informed consent. Edits to the fourth paragraph: The study will start with a screening ~~visit followed by a screening~~ period of up to 60 days prior to harvest (Visit 2: Assessment B). The screening visit (Visit 1~~, Day -33~~) is defined as the visit during which the informed consent form is signed, before any study-related procedures. Edits to the third bullet in the list of screening visit parameters: Confirmation of ADA-SCID (if not performed before the screening period) Duplicate ADA-SCID confirmation paragraph has been removed	
5.1.2 Autologous Bone Marrow Harvest Period (Visit 2) Bone Marrow Harvest (Assessment B)	For BM harvesting, a central venous access device will be required (if not already in place) for ease of phlebotomy and drug administration. The decision between PICC line, a tunneled central venous catheter, or an implanted subcutaneous access device will be made by discussions with subjects parents and line placement surgeon. A PICC line or central venous line may instead be placed as a separate procedure with appropriate sedation or during the general anesthesia for the BM harvest. The harvest procedure will involve a bilateral BM aspiration in the posterior iliac crests, where a total volume required should be between 15-20 ml/kg of the subjects body weight. The BM sample will be directly transported to the GMP manufacturing location. Compatible irradiated PRBC (10 to 15 cc/kg) may be transfused following the completion of marrow harvest, if considered appropriate by the Investigator.~~This harvesting procedure should be followed by the transfusion of PRBC.~~ If the subject is otherwise well, he/she may be discharged to home, 1-2 days after harvest. If the bone marrow harvest fails to collect sufficient HSCs for transplantation, the Investigator may perform a second harvest according to local protocols. Any AEs and concomitant medication use should be recorded.	5

Section No./Title	Revision	Relates to Change No.
	Backup Sample A back up sample will be taken in the event of damage during thawing or lack of engraftment at 42 days post GTMP (see Section 3.4), and must constitute 5 x $10e^7$ mononuclear cells/kg or 3 x $10e^7$ total nucleated cells/kg. The backup sample will be cryopreserved, and CD34+ cells will be isolated from the remainder. The backup harvest will consist of non-transduced cells that will be retained if required.	
5.1.3 Baseline (Visit 3) Pre-Conditioning (Assessment C)	Bullet nine, text added: Please note: the above immune function tests can be performed at any point after provision of consent/assent and prior to busulfan administration in younger subjects if deemed appropriate by the Investigator. Bullet ten, text added: Peripheral blood mononuclear cells (PBMC) and serum banking for RCL determination. These test can be performed at anytime following BM harvest (Visit 2: Assessment B) and prior to OTL-101 administration (Visit 3: Assessment F) in younger subjects if deemed appropriate by the Investigator. Bullet eleven, text added: Measurements of erythrocyte ADA enzymatic activity and erythrocyte deoxyadenine nucleotide levels. These test can be performed at anytime following BM harvest (Visit 2: Assessment B) and prior to OTL-101 administration (Visit 3: Assessment F) in younger subjects if deemed appropriate by the Investigator. Second paragraph deleted: ~~If the OTL-101 product does not meet the release criteria (see Section 3.7.1), the subject may be readmitted once again for a further harvest procedure, if this is deemed appropriate by the Investigator.~~	1
5.1.7 Follow-Up (Visits 4 to 11)	Gene transduction/expression edited replacing RCL with VCN at Month 3, Month 6, Month 12, Month 24 and early termination visits:	4

Section No./Title	Revision	Relates to Change No.
	Measurement of the proportion of cells containing the inserted *ADA gene* in PBMC and granulocytes as well as FACS-sorted T-cells and myeloid cells by PCR for ~~RCL~~VCN	
5.1.7 Follow-Up (Visits 4 to 11)	Gene transduction/expression bullet, text removed from Month 1, Month 3, Month 6, Month 9,and Month 12; Measurement of ADA expression in erythrocytes by ADA enzymatic assay ~~and possibly reverse transcriptase (RT) PCR (if samples are positive by DNA-PCR)~~ and deoxyadenine nucleotides in RBC.	4
5.1.7 Follow-Up (Visits 4 to 11) Day 42 (Visit 5 - +/- 1 week)	Day 42 assessment will use laboratory parameters to confirm if immune reconstitution has occurred. If this has not happened by Day 42 the backup HSC sample will be processed according to instructions in Section 3.7. If the Investigator consider the use of the back up HSC sample is not in the best interests of the subject, rescue medication, as per standard institute protocols, will be implemented. Any treatment administered to the subject will be recorded in the eCRF. Adverse events will also be recorded.	5
5.1.7 Follow-Up (Visits 4 to 11) Month 6 (Visit 7 - +/- 2 weeks)	Fourth bullet edited: Assessment of the success of treatment at the subject level ("responder analysis") at 6 months post GTMP administration, by evaluating: a) Evidence of erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units), b) Evidence of hematopoietic~~immune~~ reconstitution (absolute number of CD3 cells ≥200/mm3), c) Detectable gene-marked granulocytes by differential polymerase chain reaction (dPCR)/qPCR (≥1/10,000 cells). If there is no evidence of hematopoietic reconsitution, subjects will require PEG-ADA and/or allogenic HSCT, if available, and deemed appropriate by the Investigator. The definition applied for lack of hematopoietic reconstitution is the failure to meet ALL three criteria listed above (a-c). ~~If a subject fails to meet all~~Any subject not meeting these three criteria~~, they~~ will be deemed a treatment failure and should be withdrawn from the study as described in	5

Section No./Title	Revision	Relates to Change No.
	Section 3.6.1.	
5.1.7 Follow-Up (Visits 4 to 11)	Immune function test bullet, third sub-bullet text added t Month 12, Month 18, Month 24 and early termination visits: Measurement of specific antibodies to tetanus toxoid only if subjects have stopped immunoglobulin replacement and have received tetanus vaccination	4
6.1.1 Autologous Bone Marrow Harvest and Processing	Section removed: ~~In the US, because of the early detection of the disease through the screening of newborns, bone marrow will be used as the source of CD34+ HSCs for the manufacture of OTL-101.~~ ~~If the subject does not already have a central venous access device, a central venous access device will be placed for ease of phlebotomy and drug administration. The decision between PICC line, a tunneled central venous catheter, or an implanted sub-cutaneous access device will be made by discussions with parents and line placement surgeon. A PICC line may instead be placed as a separate procedure with appropriate sedation or a central venous line may be placed during the general anesthesia for the bone marrow harvest.~~ ~~Bone marrow will be harvested from the subject under general anesthesia from the posterior iliac crests on both sides by multiple punctures. The amount of marrow collected will be equivalent to 15 to 20 ml/kg of body weight and will be directly transported to the GMP manufacturing location. Compatible irradiated PRBC (10 to 15 cc/kg) will be transfused at the completion of marrow harvest.~~	4
6.1.1 Administration of OTL-101 Final Product Dose and Infusion Volume Calculations	Section edited: Dose calculation forms are provided in Section 17: Appendix 1. Use the Single Product Bag form unless there is more than one final product bag. Use the Multi-Bag form only if there are two product bags. A form must be completed for each subject and will be used as source data. This form should be filed in the subject's medical notes. *Dose calculation*: 1. Prior to busulfan administration, calculate the total Final Product (FP) CD34+ cells per kg	7

Section No./Title	Revision	Relates to Change No.
	as described on the appropriate worksheet. All values required to perform the calculation will be found on the Certificate of Analysis for the OTL-101 Final Product. 2. Select course of action based on the following criteria: • The **Total FP CD34$^+$ cells per kg** is less than 2.0x10^6 CD34$^+$/kg. An additional final product manufacturing process will be required to obtain the minimum acceptable dose • The **Total FP CD34$^+$ cells per kg** is greater than 2.0x10^6 CD34$^+$/kg and less than 20.0x10^6 CD34$^+$/kg. Thaw and infuse the entire combined final product volume • The **Total FP CD34$^+$ cells per kg** is greater than 20.0x10^6 CD34$^+$/kg, The volume to infuse must be calculated as described on the appropriate worksheet. Do not rinse the partially infused OTL-101 bag after infusion. The final dose and volume administered to the subjects is required to be recorded in the eCRF. When more than one bag is used, only infuse one OTL-101 product bag per hour. ~~*Dose calculation*~~: ~~3. Calculate the Final Product CD34+ cells per kg by multiplying the Final Product Total Cells by the % CD34+ Purity and then dividing by the subject Weight (obtained at harvest). Obtain these values from the Final Product certificate of analysis:~~ ~~Final Product Total cells~~ ~~x 10^6~~ ~~% CD34 Purity~~ ~~÷~~ ~~Subject weight~~ = ~~Final Product CD34 cells / kg~~ ~~10^6/ kg~~ ~~4. Select course of action based on the following criteria:~~ ~~• The Final Product CD34$^+$ cells per kg is less than 2.0x10^6 CD34$^+$/kg. An additional final~~	

Section No./Title	Revision	Relates to Change No.
	~~product manufacturing process will be required to obtain the minimum acceptable dose~~ ~~– The Final Product CD34^{+} cells per kg is greater than 2.0x10^{6} CD34^{+}/kg and less than 20.0x10^{6} CD34^{+}/kg. Thaw and infuse the entire final product volume~~ ~~– The Final Product CD34^{+} cells per kg is greater than 20.0x10^{6} CD34^{+}/kg, calculate the volume to infuse as follows and do not rinse the OTL-101 bag after infusion:~~ ~~a) Obtain the Final Product Certificate of Analysis which contains the values for the Final Product (FP) Total Cells, %CD34+ Purity, and Final Product Volume~~ ~~b) Calculate the Final Product CD34+ cells/mL by multiplying the Final Product Total Cell Number by the %CD34+ Purity and dividing by the Final Product volume~~ ~~x 10^{6} / Final product total cells = % CD34 Purity / Final product CD34 cells x 10^{6} ÷ Final product volume (mL) = Final product CD34 cells/mL~~ ~~Calculate the infusion volume by dividing 20.0x106 CD34+/kg by the FP CD34+ cells/mL.~~ ~~20.0x10^{6} CD34^{+}/kg ÷ x10^{6}CD34^{+}/mL = Final product CD34^{+} cells/mL / Infusion volume (mL)~~ ~~The final dose and volume administered to the subjects is required to be recorded in the eCRF.~~	
6.2.1.1 Transplant-related Therapies Enzyme replacement	2. Rules for re-starting ERT or plan allogenic HSCT (if available) If, by 6 months after the cell product infusion, there is no evidence of hematopoietic reconsitution following the infusion of the back up sample and rescue medication,~~as defined below~~, subjects will require rescue PEG-ADA or allogenic HSCT if available. Positive evidence	5

Section No./Title	Revision	Relates to Change No.
therapy with PEG-ADA	of hematopoietic reconstitution will require: a. RBC ADA >0 Units, b. absolute CD3+ T-cell counts ≥200/mm^3, and c. peripheral blood samples positive for vector sequences by qPCR (>1/10,000 cells). In addition, PEG-ADA may be re-started if the Investigator deems it in the best interests of the subject on clinical grounds, e.g. multiple, serious or unresponsive infections or sub-normal immune reconstitution. Once ERT has been re-started, the subject will remain in the study and will continue to be followed-up. If the subject fails to demonstrate hematopoietic reconsitution and has the possibility of receiving a transplant, the subject should be withdrawn from the study in order to follow institute transplantation protocols.	
7.2 Efficacy Assessments Quality of Life	Sentence added to the end of the last paragraph: Questions suitable/relevant to the age of the subject will be posed.	4
8.6.3 Urinalysis	Text removed: Fresh urine samples ~~(at least 10 mL)~~ will be collected and will include the assessment of parameters listed in Table 3, according to the times indicated in Table 1 and Table 2	4
9.5.2 Full Analysis Set	Text added: The full analysis set will be identical in composition to the safety analysis set. Subjects not receiving the product due to failure of the manufacturing process will be part of Intent To Treat group.	9
17 Appendices Appendix 1: OTL-101 Infusion Procedure and Dosing Forms	First bullet added to “Infusion Procedure”, subheading “General” Obtain and review the Dose and Infusion Volume Calculation Worksheet which was completed prior to Busulfan administration, Third bullet, last sentence edited in “Infusion Procedure”, subheading “Infusion”	7

Section No./Title	Revision	Relates to Change No.
	~~Do not thaw subsequent bags until the previous bag's infusion is complete~~ Only one OTL-101 product bag may be infused per hour, Fourteenth bullet, sentence added in "Infusion Procedure", subheading "Infusion" Do not infuse more than one OTL-101 product bag per hour. First bullet edited in "Infusion Procedure", subheading "Post Infusion" Resume the normal saline infusion for 30 minutes to ensure that no product remains in the IV catheter tubing The following worksheets (regarding dose calculation for single and multi-product bag infusion) and accountability log were added to Appendix 1: Final Product Dose and Infusion Volume Calculation – Single Product Bag Final Product Dose and Infusion Volume Calculation – Multi Product Bag Global Investigational Product Accountability Log	
Entire document	1. Clarity on the use of bone marrow as the source of hematopoistic stem cells. 2. Hematopoietic reconstitution replaces immune reconstitution. 3. Spelling corrections and abbreviations.	4

PROTOCOL AMENDMENT 2: OTL-101-4 (05 MAY 2017)

EFFICACY AND SAFETY OF A CRYOPRESERVED FORMULATION OF AUTOLOGOUS CD34+ HEMATOPOIETIC STEM CELLS TRANSDUCED EX VIVO WITH EFS LENTIVIRAL VECTOR ENCODING FOR HUMAN ADA GENE IN SUBJECTS WITH SEVERE COMBINED IMMUNODEFICIENCY DUE TO ADENOSINE DEAMINASE DEFICIENCY

STUDY PROTOCOL
STUDY number: OTL-101-4
PRODUCT: A CRYOPRESERVED FORMULATION OF AUTOLOGOUS CD34+ HEMATOPOIETIC STEM CELLS TRANSDUCED EX VIVO WITH EFS LENTIVIRAL VECTOR ENCODING FOR THE HUMAN ADA GENE (OTL-101)
[IND number: 15440]

Original Protocol, Version 1.0: 24 October 2016
Protocol Amendment 1, Version 2.0: 18 January 2017
Protocol Amendment 2, Version 3.0: 05 May 2017

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY
STUDY number: OTL-101-4
Protocol Amendment 2, Version 3.0: 05 May 2017

RATIONALE AND SUMMARY OF CHANGES

The purpose of protocol amendment 2 (05 May 2017) is to align the protocol with the statistical analysis plan and provide flexibility in the timelines for administration of the Busulfan-conditioning regimen reflecting current clinical practices.

This document will summarise the key changes accordingly:

1. Align the protocol with the statistical analysis plan and apply consistency across objectives and endpoints.
2. Provide flexibility in the timelines for administration of the Busulfan-conditioning regimen reflecting current clinical practices and screening assessment windows.
3. Change in CRO contact details.
4. Clarification of back up sample timings and storage requirements of the OTL-101 product.
5. Revision of multi-bag infusion form.
6. Administrative changes and typographical errors (which are not shown in the table below).
    - Change all references of gene therapy medicinal product (GTMP) or protocol treatment to OTL-101 throughout the document,
    - Update of subject recruitment figures for existing studies as of 14 October 2016,
    - Readability,
    - Correct typographical errors

PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale. Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

<table>
<tr><th>Section No./Title</th><th>Revision</th><th>Relates to Change No.</th></tr>
<tr><td>[redacted]</td><td></td><td>3</td></tr>
<tr><td>Table 1: Schedule of Events – Screening to Day 24</td><td>
<table>
<tr><td>Study Period</td><td colspan="2">Conditioning</td></tr>
<tr><td>Visit</td><td>3</td><td>3</td></tr>
<tr><td>Assessment</td><td>D</td><td>E</td></tr>
<tr><td>Day</td><td>Day -4 to -3</td><td>Day -2 to -1</td></tr>
</table>
</td><td>2</td></tr>
<tr><td>2.2.1 Primary Study Objectives</td><td>First primary objective:
This 6 month data will be used to compare the proportion of “responders ~~analysis” obtained from~~among subjects treated with the cryopreserved population with that in a comparable population obtained from one of the ongoing Phase I/II studies using the fresh formulation</td><td>1</td></tr>
<tr><td>3.1 General Design and Study Scheme</td><td>Second paragraph:
The aim of this clinical study is also to assess the success of treatment at the subject level (“responder analysis”) 6 months post OTL-101 infusion~~GTMP administration~~, using predictive criteria for overall survival and event free survival and to compare this with data obtained from one of the ongoing Phase I/II clinical studies using the fresh formulation of OTL-101.
Sixth paragraph, first bullet, third sentence:
This data will be compared with available data from one of the ongoing Phase I/II studies ~~UCLA ongoing Phase I/II study, treated with~~using the OTL 101 fresh formulation</td><td>1</td></tr>
<tr><td>3.1 General Design and Study</td><td>Figure 1 updated to reflect the revised schedule for conditioning as specified in Table 1: Schedule of Events – Screening to Day 24</td><td>2</td></tr>
</table>

Section No./Title	Revision	Relates to Change No.
Scheme		
3.2.1 Primary Efficacy Evaluations and Endpoints And 7.1.1 Primary Efficacy Endpoints	The primary efficacy endpoints for this study include: 1. Evaluation of OTL-101 at 6 months, post OTL-101 infusion~~GTMP administration~~, in support of CMC assessing comparability of cryopreserved and fresh formulations, by evaluating~~evaluation of~~ the success of therapy ~~success~~ for each subject based on all three ~~the following parameters~~ listed below and their thresholds being met: a) Erythrocyte ADA enzyme activity above baseline/pre-treatment level ~~Red blood cells ADA~~ (>0 Units), b) Absolute CD3+ T-cell counts ≥200/mm3, and c) Peripheral blood samples positive for vector sequences by qPCR(≥1/10,000 cells). Please note: ~~subjects must meet all three criteria.~~ Subjects not meeting any ~~these~~ one criterion~~a~~ will be designated a failure (non-responder). Further details are described in Section 3.6.1. This data will be ~~used to~~ compared with subject data from the ~~ongoing~~ Phase I/II study using the fresh formulation.	1
3.2.3 Safety Evaluations and Endpoints	The safety and tolerability of OTL-101 will be assessed throughout the study by evaluating adverse events (AEs), clinical laboratory test results, vital signs measurements, electrocardiogram (ECG), physical examination results, and concomitant medication usage. In addition, the safety of OTL-101 will be assessed through monitoring infections and any emergence of replication competent lentivirus, monoclonal expansion or leukemia due to chosen vector.	1
3.2.4 Exploratory Endpoints and Evaluations	The exploratory endpoints will include laboratory correlates of efficacy that will be used to assess the level of gene correction, engraftment and immune reconstitution ~~as exploratory endpoints.~~: • Quantification of clonal diversity of vector integrants. • TREC and FACS for TCR V-beta family use. • Percentage gene marking in peripheral blood cells. • Adenosine deaminase enzyme activity in erythrocytes. • Total adenine nucleotides in erythrocytes. • Vector integration analysis.	1

Section No./Title	Revision	Relates to Change No.
	• Immune reconstitution o Absolute Lymphocyte Count. o Absolute numbers of T, B, NK lymphocytes in peripheral blood. o T lymphocyte proliferative responses to mitogen (PHA) and to antigens (tetanus toxoid after vaccination) o Serum immunoglobulin levels (IgG, IgA and IgM). ~~Full descriptions of the exploratory assessments are presented in Section 7.1.3.~~	
3.7.1 OTL-101 Storage and Security	~~The protocol treatment being investigated in this study (~~OTL-101~~)~~ is manufactured from the individual subject's bone marrow CD34+ HSCs. The Subject's BM will be harvested at the clinical site and then transferred to a GMP compliant manufacturing facility for onward processing. At the GMP facility CD34+ cells will be isolated, transduced, formulated, filled into cryobags and then cryopreserved. The autologous, cryopreserved OTL 101 ~~protocol~~ treatment will be stored as part of a controlled inventory at ≤-135°C in the vapor phase of LN2 until released for administration to the subject. The storage of OTL-101 will be in accordance with UCLA standard procedures. Access to OTL-101 will only be permitted for individuals designated by the Investigator who are responsible and trained staff listed in the site personnel log at the clinical site. For release, the product must meet defined acceptance criteria for Quality Control assays of safety, identity, viability, purity and potency. During the first harvest, ~~A~~a backup ~~harvest~~ sample of non-transduced CD34+ cells will be retained in i) the event of product damage or below product release specifications ~~during the thawing~~ of the ~~GTMP~~ OTL-101 that would prevent the infusion ~~to~~ taking~~e~~ place even~~of the GTMP al~~though the patient has already been conditioned or ii) lack of engraftment/hematopoeitic reconstitution at 42 days post ~~GTMP~~ OTL-101 infusion (see Section 3.4). In the event that a second BM harvest is required, the same storage and security procedures will be followed. A second backup sample is only required if the first backup is not reliable, e.g. if contamination of the initial marrow harvest had occurred.	4
3.7.2 OTL-101 Production	First bullet: NOTE: On Day 1, a back-up fraction of mononuclear cells will be cryopreserved . This back-up will constitute ≥3~~5~~.0 x 10e7 mononuclear cells/kg ~~OR 3.0 x 10e7 total nucleated cells/kg~~. Third bullet, sub-bullet iii:	4

Section No./Title	Revision	Relates to Change No.
	Temperature has been added at ≤-135°C.	
3.7.3 OTL-101 Administration	Third bullet: Hold the shipping container in secure storage at ≤-135°C , according to UCLA standard procedures, until requested by the Investigator. Storage instructions are specified in Section 3.7.1. Penultimate paragraph, sentence added: If more than one bag is needed to achieve the dose, the weight of the subject must be higher than 4 kg to comply with recommended limit of DMSO per kg body weight per day prior to infusion.	4 and 5
3.7.4 OTL-101 Accountability	Second paragraph, last sentence: The OTL-101 ~~protocol~~ treatment must~~is~~ not ~~to~~ be ~~administered or dispensed to~~infused to any person who is not ~~a study subject under this protocol.~~the biological owner of the stem cells.	4
5.1.1 Screening (Visit 1)	First paragraph, third sentence: Evaluations obtained as part of routine medical care and performed during the screening period may be used in place of the study specific evaluations if performed within 60 days of the harvest visit (Visit 2: Assessment B), except for cytogenetics, which may be used independently from the time they have been performed. List of screening assessment, blood tests, fourth bullet: Peripheral blood or bone marrow for cytogenetic analysis (if cytogenetic testing has not been performed on cells from amniocentesis) by karyotype, Comparative Genome Hybridization (CGH), whole exome sequencing (WES) or other and may be used independently from the time they have been performed.	2
5.1.2 Autologous Bone Marrow Harvest Period (Visit 2)	Fifth paragraph: If the first bone marrow harvest fails to collect sufficient HSCs for transplantation or if the product fails to meet the~~any~~ release criteria, the Investigator may perform a second harvest ~~according to local protocols~~. Screening studies will not be repeated unless 1 year or more has passed from the time of the first harvest. Backup Sample sub-heading: A back up sample will be taken at the first harvest procedure, in the event of damage during thawing, failure to meet product release specifications or lack of engraftment at 42 days post	2 and 4 4

Section No./Title	Revision	Relates to Change No.
	~~GMTP~~OTL-101 infusion (see Section 3.4), and must constitute ≥3.0~~5~~ x $10e^{7}$ mononuclear cells/kg ~~or 3 x $10e^{7}$ total nucleated cells/kg~~ The backup sample will be cryopreserved, and CD34+ cells will be isolated from the remainder. The backup harvest will consist of non-transduced cells that will be retained if required. A second backup sample is only required if the first backup is not reliable, e.g. if contamination of the initial marrow harvest had occurred.	
6.1.1 Administration of OTL-101	Last paragraph edited: When more than one bag is ~~used~~needed, only infuse one OTL-101 product bag per hour, and ensure prior to infusion that the weight of the subject is higher than 4 kg to comply with recommended limit of DMSO per kg body weight per day.	5
6.2.1.1 Transplant-related Therapies, Non-myeloablative conditioning with Busulfan	Any reference to Day -3 has been changed to Day-4 to -3. Any reference to Day -1 has been changed to Day -2 to -1	2
6.3 Administration Compliance	Second sentence added: If a subject fails to demonstrate engraftment/hematopoietic reconstitution at 42 days post initial OTL-101 infusion, their backup sample may be administered.	4
7.1.1 Primary Efficacy Endpoints	Last paragraph: The proportion of responders in this study~~is data~~ will be ~~used to~~ compared with the proportion ~~subject data from~~ of responders in one of the Phase I/II ongoing studies using the fresh formulation	1
7.1.3 Exploratory Efficacy Endpoints And 9.3.3 Exploratory	Removal of timepoints for the assessment of exploratory endpoints.	1

Section No./Title	Revision	Relates to Change No.
Efficacy Endpoints		
Section 9	The presentation of the study endpoints has been re-ordered.	1
~~9.3.2~~ 9.3.1 Primary Efficacy Endpoints	The primary efficacy endpoints for this study include treatment success at 6 months, post OTL-101 infusion, and overall survival and event-free survival at 12 months post OTL-101 infusion. Treatment success at 6 months, post OTL-101 infusion, will be performed in support of CMC assessing comparability between cryopreserved and fresh formulations. Evaluation of the success of therapy will be based on the following parameters and their thresholds: a) Erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units), b) Absolute CD3+ T-cell counts ≥ 200/mm3, and c) Peripheral blood samples positive for vector sequences by qPCR(≥1/10,000 cells). Treatment will be deemed sucessful in subjects meeting all three above thresholds. These subjects will be designated as "responders". Those subjects failing to meet at least one of the above thresholds will be considered to be "non-responders." ~~The primary efficacy endpoints of the study include overall survival and event-free survival at 12 months post OTL-101 administration.~~ Overall Survival is defined as the time-to death from ~~the start of protocol treatment~~OTL-101 infusion if death occurred, or to the date of the last evaluation~~furthest point observed~~ if death did not occur. If no death occurred, the subject's data will be considered censored ~~from~~ at the last ~~observed time point~~evaluation. Event Free Survival is defined as the time-to event from ~~the start of protocol treatment~~ OTL-101 infusion if an event occurred, or to the date of the last evaluation~~furthest point observed~~ without an event if no event occurred. If no event occurred, the subject's data will be considered censored ~~from~~ at the last evaluation~~observed time point~~. Event is defined as any of the following: • Death • Returning to PEG-ADA ERT • Need of rescue HSCT ~~In addition, to allow the evaluation of GTMP OTL-101treatment at 6 months post administration in support of CMC assessing comparability between cryopreserved and fresh~~	1

Section No./Title	Revision	Relates to Change No.
	~~formulations, evaluation of the success of therapy success will be based on the following parameters and their thresholds will be performed:~~ ~~a) Red blood cells ADA >0 Units,~~ ~~b) Absolute CD3+ T-cell counts ≥ 200/mm3, and~~ ~~c) Peripheral blood samples positive for vector sequences by qPCR(≥1/10,000 cells).~~ ~~Subjects meeting all three thresholds will be designated "success" and those failing to meet at least one of the thresholds will be designated "failure."~~	
9.5 Analysis Populations	Analysis sets has been changed to analysis populations throughout.	1
~~9.5.2~~ 9.5.1 ~~Full Analysis Set~~Efficacy Population	The efficacy population will be a modified intent-to-treat population including all subjects treated with OTL-101 within this protocol~~full analysis set will be identical in composition to the safety analysis set. Subjects not receiving the product due to failure of the manufacturing process will be part of Intent To Treat group.~~	1
9.6.1 Overview	Second paragraphs onwards: ~~Data listings by subject will be provided and, where applicable, presented graphically~~. All statistical analyses will be performed ~~and data appendices will be created~~ using Statistical Analysis System (SAS)® Version 9.4. or higher. ~~Three analyses will be performed during this study as summarized in Section 9.6.5. At the same time, subject disposition, baseline characteristics and safety analysis will also be performed at these three stages.~~ *The text below has been moved from Efficacy analysis section and revised.* Statistical analyses will be performed, and their outcomes presented, in three stages: 1. The first (interim) analysis will compare the success/failure data 6-months post OTL-101 infusion. The success/failure of the OTL-101 cryopreserved formulation will be defined by the three criteria listed in the primary objectives (Section 2.2.1). This data will be compared with available data from the ongoing UCLA Phase I/II study, treated with using the OTL 101 fresh formulation. This will support the CMC comparability data between OTL-101 cryopreserved and fresh formulations. Secondary and exploratory endpoints will also be described. 2. The second (primary) analysis will determine the 12 month overall survival and event	1

Section No./Title	Revision	Relates to Change No.
	free survival for all subjects. Secondary and exploratory endpoints, as well as baseline characteristics, subject disposition, and safety data will also be described. 3. The third (and final) analysis will be performed to determine the overall survival and event free survival for all subjects 24 months post OTL-101 infusion. Secondary and exploratory endpoints, as well as baseline characteristics, subject disposition, safety and Busulfan PK data will also be described. This study is not designed to be powered to demonstrate statistical significance; therefore, no correction for overall Type I Error will be performed.	
9.6.2 Subject Disposition	Subject disposition will be tabulated; the number of enrolled, exposed, prematurely terminated, ongoing and completed subjects will be summarized ~~by visit (where relevant)~~.	1
9.6.4 Efficacy Analyses	The primary efficacy endpoints will be summarized as follows: Responder Analysis The proportion of responders in this study will be summarized and compared with the proportion of responders in the Phase I/II ongoing studies using the fresh formulation. Overall and Event Free Survival Overall and event free survival will be summarized using: • Proportion of patients who survived at each specified analysis timepoint, with the exact binomial 95% confidence interval. • Kaplan-Meier survival curve of time to death/event. All efficacy summaries will be descriptive, there will be no formal statistical comparisons.	1
~~9.6.4~~ 9.6.5 Safety Analyses	All AEs will be coded according to the current Medical Dictionary for Regulatory Activities (MedDRA) version and will be classified by MedDRA preferred term and system organ class. AE listings will be presented by subject, system organ class and preferred term. Incidence of treatment emergent AEs (TEAE) and SAEs will be tabulated by system organ class and preferred term. In addition, summary tables will be presented by maximum severity, relationship to treatment and TEAEs associated with premature withdrawal from the study. A TEAE is defined as any AE that occurs during the active phase of the study if: • it was not present prior to receiving the OTL-101 infusion, or • it was present prior to receiving the OTL-101 infusion but the intensity increased during the active phase of the study, or	1

Section No./Title	Revision	Relates to Change No.
	• it was present prior to receiving theOTL-101 infusion, the intensity is the same but the drug relationship became related during the active phase of the study. Summary statistics (mean, median, SD and range as appropriate) will be presented for vital signs, ECG parameters and, clinical laboratory tests at each assessment along with change from baseline. For laboratory data, abnormal values will be flagged in the data listings. Shift tables will be presented of the number and percentage of subjects with low, normal or high values and normal or abnormal examinations at each visit and overall. ~~Safety will be analyzed descriptively over time. Coding will be performed using CTCAE MedDRA Version 4.0 or higher. Adverse events, including SAE's, will be presented by:~~ ~~• System organ class and preferred term~~ ~~• Relation to autoimmunity~~ ~~• Whether or not the event led to administration of PEG-ADA, use of immunoglobulin replacement therapy, high-dose steroids, or infusion of additional cells (back-up cells)~~ ~~Where appropriate, shift tables will be presented for vital signs and physical examination.~~	
9.7 Pharmacokinetic Analysis	Second paragraph, first sentence: The following Busulfan PK parameters will be ~~examined~~ summarized~~and will be graphically presentated for each subject (concentration versus time).~~	1
17: Appendix 1	Replacement of Final Product Dose and Infusion Volume Calculation – Multi Product Bag	5

IND 15440
Study no.: OTL-101-4

Summary of protocol changes
Study protocol version 3.0, dated 05 May 2017
Updated document: Study Protocol Version 4.0, dated 08 September 2017

- **Page 4, Synopsis, and Section 3.1, General Design and Study Scheme** – version 3.0 of the protocol included language regarding administration of enzyme replacement therapy (ERT). This was modified to clarify that ERT use and discontinuation is to be evaluated in treated patients at the 1 month follow-up visit. Additionally, the text was updated to clearly state that the need to reinstate ERT will be assessed at all follow-up visits after month 1. The windows for all study visits were clearly stated in the synopsis, and the window for ERT discontinuation was modified from (Day 30 plus or minus 3 days) to (Day 30 minus 3 plus 15 days), allowing the Investigator to delay ERT discontinuation by a few days if in the best interest of the subject;
- **Page 5, Synopsis; Section 3.1, General Design and Study Scheme; Section 3.6.1, Subject Stopping Rules and Criteria for Discontinuation; Section 5.1.7, Follow-up (Visits 4 to 11); Section 6.1.2, Rescue Administration of Autologous HSC Backup -** hematologic reconstitution at Day 42 was more clearly defined throughout the text, and the parameters used to define a failure of hematologic reconstitution at Day 42 were simplified according to the Investigator's clinical judgement;
- **Pages 9, 11 and 12, Schedule of Events** – changes were made to the assessments required, their schedule and the legend. The "TREC and TCR Vβ" and "IgG, IgA, IgM levels" assessments were removed to avoid repetition, as already contemplated under "immune function". The "Presence of ADA gene in PBMC/granulocytes" assessment was updated to include the possibility of being carried out in lineage-sorted cells. The "RCL assay in PBMC" assessment was added, and the schedules of the "ADA enzymatic assay" and "Deoxyadenine nucleotides in RBC" assessments were updated, in order to collect necessary safety data and data on treatment effect and patient status. Flow cytometry markers in the legend of the schedule of events were updated to match those described in the body of the protocol, and footnotes were updated and rearranged for clarity;
- **Section 3.6.1, Subject Stopping Rules and Criteria for Discontinuation** – this section was updated following confirmation of treatment failure for patient 602, upon suggestion by the FDA;
- **Section 5.1.7, Follow-up (Visits 4 to 11)** – the text was modified to reflect the changes made in the schedule of events;
- Minor readability and typographical errors were corrected in the text (not shown in the table below).

Section	Previous wording (v3.0)	New wording (v4.0)	Rationale
Synopsis, Study Design (page 4)	A failure of hematologic reconstitution is defined as at least two of the following: absolute neutophil count (ANC) <200/mm^3, platelets <20,000/mm^3 without transfusions, hemaglobin (Hb) <8.0 g/dl without transfusions on three independent and consecutive determinations over at least 10 days beyond Day 42 from initial OTL-101 infusion.	A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of OTL-101 infusion.~~at least two of the following: absolute neutophil count (ANC) <200/mm^3, platelets <20,000/mm^3 without transfusions, hemaglobin (Hb) <8.0 g/dl without transfusions on three independent and consecutive determinations over at least 10 days beyond Day 42 from initial OTL-101 infusion.~~	The parameters used to define a failure of hematologic reconstitution were simplified according to the Investigator's clinical judgement.
Synopsis, Study Design (Page 4)	For subjects who have successfully received the OTL-101 product, PEG-ADA ERT will be discontinued at Day+30 (+/-3) after the transplant. After their discharge from hospital, the subjects will be seen at regular intervals to review their history, perform examinations and draw blood samples at Months 1, 3, 6, 9, 12, 18, and 24. Any medically-indicated interventions will be determined at these visits. Hematopoietic reconstitution will be assessed at Day 42, and in the event of no reconstitution the backup HSC sample will be administered. Hematapoietic reconstitution will be reassessed at Month 6 visit and if there is still	For subjects who have successfully received the OTL-101 product, PEG-ADA ERT use will be evaluated at the 1 Month follow-up visit (30 days post treatment ± 7 days) and discontinued at Day 30 (-3/+15) after the transplant. Hematologic reconstitution will be assessed at Day 42 (± 7 days), and in the event of no reconstitution the backup HSC sample will be administered, if the investigator believes this is in the subject's best interest. The subjects will then be seen at regular intervals to review their history, perform examinations and draw blood samples at Months 3, 6 and 9 (all ± 2 weeks) as well as Months 12, 18 and 24 (all ± 4 weeks). Any medically-indicated interventions, including the need to reinstate PEG-ADA ERT, will be assessed at all follow-up visits after Month 1.~~For subjects who have successfully received the OTL-101 product, PEG-ADA ERT will be discontinued at Day+30 (+/-3) after the transplant. After their discharge from hospital, the subjects will be seen at~~	The language was updated for clarity and ease of reading; the ERT discontinuation window following the 1 month follow-up visit was extended, allowing the Investigator to delay ERT discontinuation by a few days if in the best interest of the subject.

	no evidence of cellular recovery, the subject will be given ERT and/or HSCT, if available, and deemed appropriate by the Investigator. After Month 24 visit, the subjects will have completed the study and may enter a long term registry.	~~regular intervals to review their history, perform examinations and draw blood samples at Months 1, 3, 6, 9, 12, 18, and 24. Any medically indicated interventions will be determined at these visits. Hematopoietic reconstitution will be assessed at Day 42, and in the event of no reconstitution the backup HSC sample will be administered~~. Hemat~~a~~opoietic reconstitution will be ~~re~~assessed again at the Month 6 visit and if there is still no evidence of cellular recovery, the subject will be given ERT and/or HSCT, if available~~,~~ and deemed appropriate by the Investigator. After the Month 24 visit, the subjects will have completed the study and may enter a long term registry.	
Schedule of Events, Legend (page 9)	CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ NK cells.	CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56/CD16+ (or CD56+/CD3-) NK cells~~.~~, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells	The flow cytometry markers were modified to match those indicated in the body of the protocol.
Schedule of Events (page 11/12)	Immune function[5] Leukemia Assessment[6]	Immune function[5] [6] Leukemia Assessment[~~6~~7]	The order of footnotes 6 and 7 was modified to account for the removal of the "IgG, IgA, IgM levels" assessment, already included in the Immune function assessment.
Schedule of Events (page 11)	*"IgG, IgA, IgM levels" assessment required at Month 1, 3, 6, 9, 12, 18, 24 and Early Termination visits*	*Line removed*	An IgG, IgA and IgM levels assessment is already included in the Immune Function assessment, so this constituted a repetition.
Schedule of Events (page 11)	*"TREC, TCR Vβ" assessment required at Month 6, Month 12, Month 18, Month 24 and Early Termination visits*	*Line removed*	A TREC and TCR Vβ assessment is already included in the Immune Function assessment, so this constituted a repetition.

Schedule of Events (page 11)	Presence of ADA gene in PBMC/granulocytes	Presence of ADA gene in PBMC/granulocytes and/or lineage-sorted cells	The header was updated to allow the investigators to also assess the presence of the ADA gene in lineage-sorted cells.
Schedule of Events (page 11)	*"FACS T-cells/myeloid by PCR for VCN" assessment required at the Month 3, 6, 12, 24 and Early Termination visits*	*Line removed*	This assessment was meshed with the previous line, adding the possibility to detect the ADA gene in lineage-sorted cells.
Schedule of Events (page 11)	*New line entered*	*The "RCL assay in PBMC" will be performed at the Month 3, 6, 12, 24 and Early Termination visits*	A line was added to clarify the Replication Competent Lentivirus assay, a required safety assay, is to be carried out at the Month 3, 6, 12, 24 and Early Termination visits.
Schedule of Events (page 11)	*The "ADA enzymatic assay" was performed at the Month 1, 3, 6, 9 and 12 visits*	*The "ADA enzymatic assay" will be performed at the Month 1, 3, 6, 9, 12, 18, 24 and Early Termination visits*	The Investigator believes the ADA enzymatic assay should also be performed at 18 months, 24 months and early termination visits to appropriately assess the effects of the treatment and the status of the patients.
Schedule of Events (page 11)	*"Deoxyadenine nucleotides in RBC" were assessed at the Month 1, 3, 6, 9 and 12 visits*	*"Deoxyadenine nucleotides in RBC" will be assessed at the Month 1, 3, 6, 9, 12, 18, 24 and Early Termination visits*	The Investigator believes the assessment of deoxyadenine nucleotides in RBC should also be performed at 18 months, 24 months and early termination visits to appropriately assess the effects of the treatment and the status of the patients.
Schedule of Events, Legend (page 12)	CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ NK cells	CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve) and CD4+/CD45RO+ (memory) T cells	The flow cytometry markers were modified to match those indicated in the body of the protocol.

Schedule of Events, Legend (page 12)	FACS=fluorescence-activated cell sorting	*Text removed*	"FACS" does not appear in the schedule of events anymore.
Schedule of Events, Legend (page 12)	VCN = vector copy number	*Text removed*	"VCN" does not appear in the schedule of events anymore.
Section 3.1, General Design and Study Scheme	A failure of hematologic reconstitution is defined as at least two of the following: absolute neutophil count (ANC) <200/mm^3, platelets <20,000/mm^3 without transfusions, hemaglobin (Hb) <8.0 g/dl without transfusions on three independent and consecutive determinations over at least 10 days beyond Day 42 from initial OTL-101 infusion.	A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of OTL-101 infusion.~~at least two of the following: absolute neutophil count (ANC) <200/mm^3, platelets <20,000/mm^3 without transfusions, hemoglobin (Hb) <8.0 g/dl without transfusions on three independent and consecutive determinations over at least 10 days beyond Day 42 from initial OTL-101 infusion.~~	The parameters used to define a failure of hematologic reconstitution were simplified according to the Investigator's clinical judgement.
Section 3.1, General Design and Study Scheme	For subjects who have successfully received the OTL-101 product, PEG-ADA ERT will be discontinued at Day+30 (+/-3) after the transplant. After their discharge from hospital, the subjects will be seen at regular intervals to review their history, perform examinations and draw blood samples at Months 1, 3, 6, 9, 12, 18, and 24. Any medically-indicated interventions will be determined at these visits. Hematopoietic reconstitution will be assessed at Day 42, and in the event of no reconstitution the backup HSC sample will be	For subjects who have successfully received the OTL-101 product, PEG-ADA ERT use will be evaluated at the 1 Month follow-up visit (30 days post treatment ± 7 days) and discontinued at Day 30 (-3/+15) after the transplant. Hematologic reconstitution will be assessed at Day 42 (± 7 days), and in the event of no reconstitution the backup HSC sample will be administered, if the investigator believes this is in the subject's best interest. The subjects will then be seen at regular intervals to review their history, perform examinations and draw blood samples at Months 3, 6 and 9 (all ± 2 weeks) as well as Months 12, 18 and 24 (all ± 4 weeks). Any medically-indicated interventions, including the need to reinstate PEG-ADA ERT, will be assessed at all follow-up visits after Month 1.~~For subjects who have successfully received the OTL-~~	The language was updated for clarity and ease of reading; the ERT discontinuation window following the 1 month follow-up visit was extended, allowing the Investigator to delay ERT discontinuation by a few days if in the best interest of the subject.

	administered. Hematapoietic reconstitution will be reassessed at Month 6 visit and if there is still no evidence of cellular recovery, the subject will be given ERT and/or HSCT, if available, and deemed appropriate by the Investigator. After Month 24 visit, the subjects will have completed the study and may enter a long term registry.	~~101 product, PEG ADA ERT will be discontinued at Day+30 (+/ 3) after the transplant. After their discharge from hospital, the subjects will be seen at regular intervals to review their history, perform examinations and draw blood samples at Months 1, 3, 6, 9, 12, 18, and 24. Any medically indicated interventions will be determined at these visits. Hematopoietic reconstitution will be assessed at Day 42, and in the event of no reconstitution the backup HSC sample will be administered~~. Hemat~~a~~opoietic reconstitution will be ~~re~~assessed again at the Month 6 visit and if there is still no evidence of cellular recovery, the subject will be given ERT and/or HSCT, if available~~,~~ and deemed appropriate by the Investigator. After the Month 24 visit, the subjects will have completed the study and may enter a long term registry.	
Section 3.6.1, Subject Stopping Rules and Criteria for Discontinuation	2. If two subjects experience prolonged unresponsive pancytopenia, defined as an initial failure of hematologic reconstitution which does not improve following the administration of the subject's autologous back-up cells. A failure of hematologic reconstitution is defined as at least two of the following: absolute neutophil count (ANC) <200/mm^3, platelets <20,000/mm^3 without transfusions, hemoglobin (Hb) <8.0 g/dl without transfusions	2. If two subjects experience prolonged unresponsive pancytopenia, defined as an initial failure of hematologic reconstitution which does not improve following the administration of the subject's autologous back-up cells. A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of OTL-101 infusion.~~A failure of hematologic reconstitution is defined as at least two of the following: absolute neutophil count (ANC) <200/mm^3, platelets <20,000/mm^3 without transfusions, hemoglobin (Hb) <8.0 g/dl without transfusions~~	The parameters used to define a failure of hematologic reconstitution were simplified according to the Investigator's clinical judgement.

	on three independent and consecutive determinations.	~~on three independent and consecutive determinations.~~	
Section 3.6.1, Subject Stopping Rules and Criteria for Discontinuation	5. Non-responders as defined in the primary endpoint criteria (Section 3.2.1), will be designated a OTL-101 treatment failure and will be withdrawn from the study to permit further treatment with PEG-ADA ERT, or HSCT if available.	If there are three ~~N~~non-responders, as defined in the primary endpoint criteria (Section 3.2.1), who will be designated an OTL-101 treatment failure and will be withdrawn from the study to permit further treatment with PEG-ADA ERT, or HSCT if available.	The language was updated following confirmation of treatment failure for patient 602, upon suggestion by the FDA.
Section 5.1.3, Baseline (Visit 3)	– Measurement of immune function: ○ Absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+, CD4+/CD45RO+	– Measurement of immune function: ○ Absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve)~~,~~ and CD4+/CD45RO+ (memory) T cells,	The language was updated for clarity.
Section 5.1.7, Follow-up (Visits 4 to 11)	– Measurement of immune function: ○ Absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ (or CD56+/CD3-) NK cells,	– Measurement of immune function: ○ Absolute numbers of CD3+, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and CD56+/CD16+ (or CD56+/CD3-) NK cells, CD4+/CD45RA+ (naïve)~~,~~ and CD4+/CD45RO+ (memory) T cells,	The language was updated for clarity, at the Month 1, 3, 6, 9, 12, 18, 24 and Early Termination visits.

	CD4+/CD45RA+, CD4+/CD45RO+		
Section 5.1.7, Follow-up (Visits 4 to 11)	Day 42 assessment will use laboratory parameters to confirm if immune reconstitution has occurred. If this has not happened by Day 42 the backup HSC sample will be processed according to instructions in Section 3.7.	The Day 42 assessment will use laboratory parameters to confirm if immune reconstitution has occurred. A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of cell infusion. If ~~this~~ hematologic reconstitution has not taken place~~happened~~ by Day 42 the backup HSC sample will be processed according to the instructions in Section 3.7~~.~~ and re-infused to overcome more severe myelosuppressive effects of the conditioning regimen. If the failure of hematologic reconstitution persist after the back-up marrow has been given through day 90 from the initial infusion of the cell product, prolonged unresponsive pancytopenia will exist.	The parameters used to define a failure of hematologic reconstitution at the Day 42 visit were clarified.
Section 5.1.7, Follow-up (Visits 4 to 11)	*New text added*	– RCL assay in PBMC,	The language was updated to reflect the change in the schedule of assessments, for the Month 3, 6, 12, 24 and Early Termination visits.
Section 5.1.7, Follow-up (Visits 4 to 11)	Serum to be banked for possible Western blot testing for RCL,	Serum to be banked for possible Western blot testing for RCL (testing performed if the RCL assay in PBMC returns a positive result),	The language was updated for clarity, for the Month 3, 6, 12, 24 and Early Termination visits.
Section 5.1.7, Follow-up (Visits 4 to 11)	Measurement of the proportion of cells containing the inserted *ADA gene* in PBMC and granulocytes as well as FACS sorted T cells and myeloid cells by PCR for VCN	Measurement of the proportion of cells containing the inserted *ADA gene* in PBMC and granulocytes and/or lineage-sorted cells~~as well as FACS sorted T cells and myeloid cells by PCR for VCN~~	The language was updated to reflect the change in the schedule of assessments, for the Month 3, 6, 12, 24 and Early Termination visits.

Section 5.1.7, Follow-up (Visits 4 to 11)	– Assessment of the success of treatment at the subject level ("responder analysis") at 6 months post OTL-101 infusion, by evaluating; a) Evidence of erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units), b) Evidence of hematopoietic reconstitution (absolute number of CD3 cells ≥200/mm3),	– Assessment of the success of treatment at the subject level ("responder analysis") at 6 months post OTL-101 infusion, by evaluating; a) Evidence of erythrocyte ADA enzyme activity above baseline/pre-treatment level (>0 Units), b) Evidence of ~~hematopoietic~~ immune reconstitution (absolute number of CD3 cells ≥200/mm3),	The language was updated for clarity.
Section 5.1.7, Follow-up (Visits 4 to 11)	*New text entered*	– Gene transduction/expression; ○ Measurement of ADA expression in erythrocytes by ADA enzymatic assay and deoxyadenine nucleotides in RBC.	The text was updated to reflect the change in the schedule of assessments, for the Month 18, 24 and Early Termination visits.
Section 6.1.2, Rescue Administration of Autologous HSC Backup	The failure of hematologic reconstitution is defined by at least any one of the following: – ANC <200/mm^3, and/or, – Platelets <20,000/mm^3 without transfusions, and/or, – Hb <8.0 g/dl without transfusions on three independent and consecutive	A failure of hematologic reconstitution is defined as persistent ANC < 200/µl or platelets < 20,000/µl on three independent and consecutive determinations over at least ten days after day 42 from the day of OTL-101 infusion. ~~The failure of hematologic reconstitution is defined by at least any one of the following:~~ ~~– ANC <200/mm^3, and/or,~~ ~~– Platelets <20,000/mm^3 without transfusions, and/or,~~	The parameters used to define a failure of hematologic reconstitution were simplified according to the Investigator's clinical judgement.

	determinations over at least 10 days, beyond Day 42 from the initial infusion of the cell product.	– ~~Hb <8.0 g/dl without transfusions on three independent and consecutive determinations over at least 10 days, beyond Day 42 from the initial infusion of the cell product.~~	
Section 7.2, Efficacy Assessments	• Serum samples will be used to detect RCL.	• Serum and PBMC samples will be used to detect RCL.	The language was updated to reflect the change in the schedule of assessments.