COVER PAGE

Official Study Title: Phase I/II, Historical Controlled, Open-label, Non-randomised, Single-centre Trial to Assess the Safety and Efficacy of EF1αS-ADA Lentiviral
Vector Mediated Gene Modification of Autologous CD34
+ Cells From ADA-deficient Individuals

Statistical Analysis Plan v12.0, 10-Jan-2018

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Statistical Analysis Plan

Study Name:	**Phase I/II, historical controlled, open-label, non-randomised, single-centre trial to assess the safety and efficacy of EF1αS-ADA lentiviral vector mediated gene modification of autologous CD34+ cells from ADA-deficient individuals**
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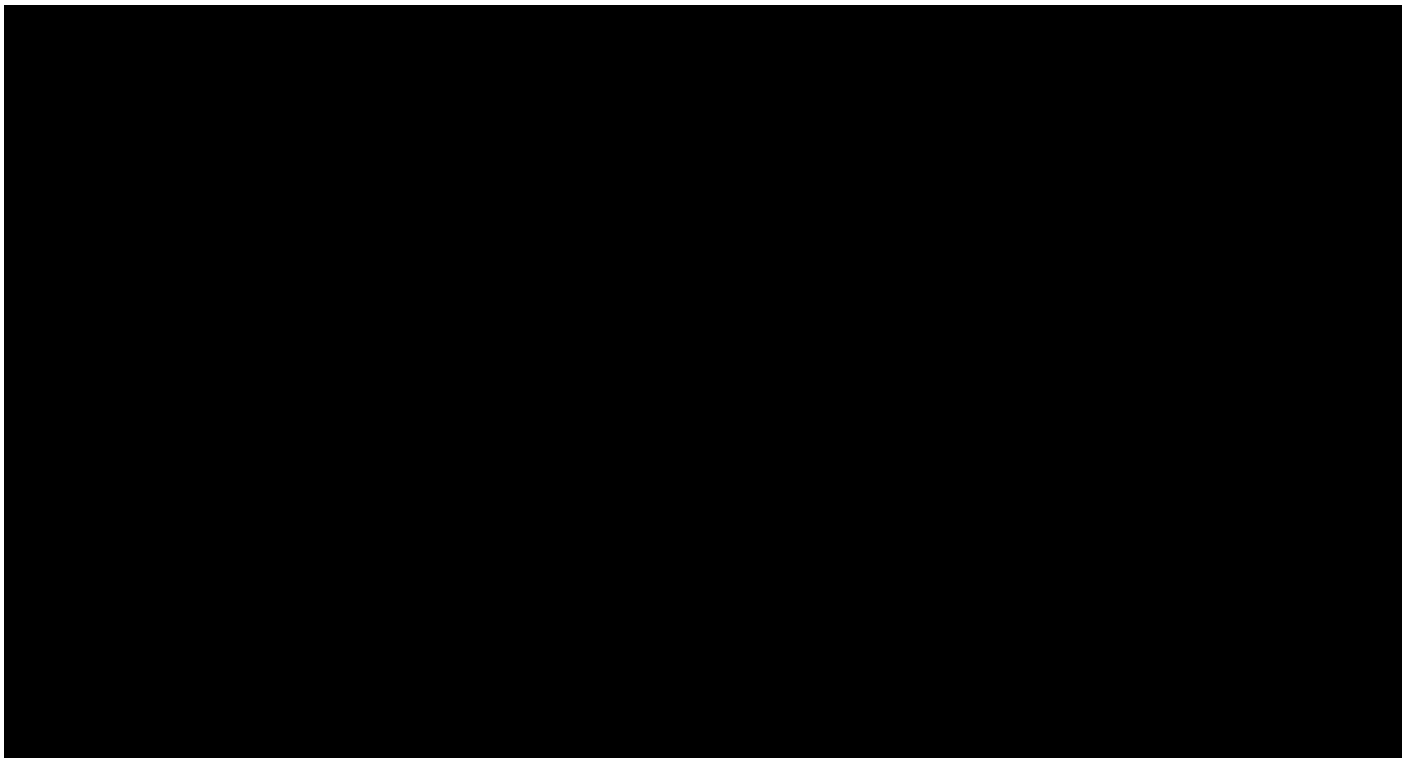

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List of Abbreviations

ADA	Adenosine deaminase
ADA-SCID	Adenosine deaminase severe combined immunodeficiency
AE	Adverse event
ALT	Alanine aminotransferase
APT	Activated partial thromboplastin
AST	Aspartate aminotransferase
AUC	Area under the curve
BM	Bone marrow
BSA	Body surface area
CI	Confidence interval
CMV	Cytomegalovirus
CUP	Compassionate use programme
dATP	Deoxyadenosine triphosphate
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
ECG	Electrocardiogram
eCRF	Electronic case report form
EFS	Elongation Factor 1α Short form
ERT	Enzyme replacement therapy
G-CSF	Granulocyte-colony stimulating factor
GFR	Glomerular filtration rate
GGT	Gamma glutamyl transferase
GOSH	Great Ormond Street Hospital
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
HSV	Herpes simplex virus
HTLV	Human T-lymphotropic virus
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgRT	Immunoglobulin replacement therapy
IMP	Investigational medicinal product
LV	Lentiviral vector
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume

MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed model for repeated measures
MNC	Mononuclear cells
NK	Natural Killer
PBMC	Peripheral blood mononuclear cells
PBSC	Peripheral blood stem cells
PCR	Polymerase chain reaction
PEG	Polyethylene-glycol
PHA	Phytohemagglutinin
PI	Principal Investigator
PT	Preferred Term
qPCR	Quantitative polymerase chain reaction
RBC	Red blood cell(s)
RCL	Replication competent lentivirus
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SCID	Severe combined immunodeficiency
SCLN	Save cells in liquid nitrogen
SOC	System Organ Class
TCR	T-cell receptor
TREC	T-cell receptor excision circle
UK	United Kingdom
VZV	Varicella zoster virus
WBC	White blood cell(s)

1 Introduction

The trial assesses a new breakthrough therapy composed of *ex vivo* genetically modified $CD34^+$ hematopoietic stem cells (HSC) for the treatment of severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID).

The investigational medicinal product (IMP) includes autologous $CD34^+$ HSC of ADA-SCID infants, children and adolescents, transduced *ex vivo* with a self-inactivating HIV-1 based lentiviral vector encoding the human ADA cDNA (EFS-ADA LV). The IMP is used as fresh cell dispersion for infusion after non-myeloablative conditioning.

2 Study Design

This is a Phase I/II, historical controlled, open-label, non-randomised, single-centre trial to assess the safety and efficacy of the IMP, i.e. autologous CD34+ HSC transduced *ex vivo* with EFS-ADA LV in ADA-SCID patients.

The IMP will be compared with an historical control group treated with allogeneic Hematopoietic Stem Cell Transplantation (HSCT), representative of standard of care.

The study is conducted in a single centre at Great Ormond Street Hospital (GOSH) for Children, London, UK.

3 Study Schedule

		Timepoint (months) ^											
	Visit window			**+/- 5d**	**+/- 5d**	**+/- 2w**	**+/- 2w**	**+/- 2w**	**+/- 2w**	**+/- 2w**	**+/- 2w**	**+/- 2w**	**+/- 2w**
Assessment	**Parameter to be reported on the eCRF**	**PreGT**	**Treatment**	**1**	**1.5**	**3**	**6**	**9**	**12**	**18**	**24**	**30**	**36**
Inclusion / Exclusion criteria	-	**X**											
Pregnancy test for patient >12 years old	-	**X***											
Diagnosis	DNA sequencing (mutation, exon, polymorphism) OR Confirmed absence of <3% enzymatic activity	**X**											
Medical history	Infection/hospitalisation history Transplantation history	**X**											
Coagulation testing	PTT, APT, Thrombin Time, Fibrinogen	**X**											
Blood ABO testing	Determination, Rhesus	**X**											
Extended B cell memory	CD19 Naïve B cells CD19+IgD+CD27- Non Switched memory B cells CD19+IgD+CD27+ Class Switched memory B cells CD19+IgD-CD27+ Transitional B cells CD19+IgM++CD38++ Plasmablasts CD19+CD38++IgMwk CD21 low B cells CD19+CD21wkCD38wk	**X**							**X**		**X**		**X**
Virology	CMV, EBV, Adenovirus, HIV1, HIV2, VZV, Hep A, Hep B, HSV, Hep C, Syphilis, HTLV1, HTLV2, Toxoplasma	**X**											
Biochemistry	Sodium, Potassium, Urea, Creatinine, Calcium, Magnesium, Phosphate, Albumin, Alkaline Phosphatase (ALP), Alanine Transaminase(ALT), gamma-glutamyltranferase(GGT), Bilirubin	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Glomerular Filtration	Weight, Creatinine,Distribution volume,Half-life Glomerular Filtration, corrected GFR	**X**											
Chest x-ray	Normal/Abnormal	**X**											
ECG	Normal/Abnormal	**X**											
Echocardiogram	Normal/Abnormal	**X**											
Lung function test if applicable\\	Normal/Abnormal	**X**											
Dental	Normal/Abnormal	**X**											
Audiology	Normal/Abnormal	**X**											
Maternal engraftment studies	Normal/Abnormal	**X**											

Assessment	Result to be reported on the eCRF	PreGT	Treatment	1	1.5	3	6	9	12	18	24	30	36
Back-up harvest	Harvest type BM or leukapheresis, CD34+cell count		X										
Harvest for IMP preparation	If leukapheresis : Mobilisation: G-CSF (date dose) +/- Plerixafor (date, dose), Leukapheresis harvest: patient's weight, date, CD34+cell dose for transduction If BM: patient's weight, date, CD34+cell dose for transduction)		X										
Conditioning	Patient weight, Busulfan dose, Busulfan AUC		X										
IMP administration	Infusion date, IMP expiry, IMP start date, IMP end date, IMP cell dose, IMP viability, IMP volume Observation before, +15min, after infusion		X										
Vital Signs	Patient's weight Temperature Heart rate Respiratory rate Oxygen saturation	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	Cardiovascular system Respiratory system Ear, Nose, Throat, Neck and Eyes (only at PreGT, 36 m) Neurological System (only at PreGT, 36 months) Abdominal Skin Other	X		X	X	X	X	X	X	X	X	X	X
Infection status	Evaluate any active infection at time of visit (respiratory tract, meningitis, sepsis, skin and mucosa, ear, nose and throat, abscess, persistent fever, other) Evaluate if there is any infection resulting in hospitalisation since last visit	X		X	X	X	X	X	X	X	X	X	X
PEG-ADA ERT status	Confirmation that if PEG-ADA has been administered or not since last visit	X		X	X	X	X	X	X	X	X	X	X
Blood Count	Haemoglobin, Platelet count White cell count Neutrophil count Lymphocyte count	X		X	X	X	X	X	X	X	X	X	X

Assessment	Result to be reported on the CRF	PreGT	Treatment	1	1.5	3	6	9	12	18	24	30	36
Lymphocyte subset counts	CD 3 CD19 CD16+ CD 56+ CD3+ CD4+ CD3+ CD8+ Naïve: CD4+CD45RA+CD27+	X		X	X	X	X	X	X	X	X	X	X
T cell function PHA stimulation**	Normal/Abnormal	X				X	X		X	X	X		
Lymphocyte function CD3 stimulation**	Normal/Abnormal								X			X	
GAM ***	Ig G level Ig A level Ig M level Immunoglobin status since last visit	X					X	X	X	X	X	X	X
TRECS	TRECS on T cells TRECS on MNCs	X					X		X		X		
Vβ Spectratyping	Reported as Normal/Abnormal						X		X	X	X	X	X
Vector copy number in cells (qPCR)	vector copy number in PBMC vector copy number in CD34 vector copy number in CD3 vector copy number in CD19 vector copy number in myeloid					X	X		X		X		X
Integration analysis	Normal/Abnormal					X****	X****		X		X****		X
Tetanus vaccination responses	Normal/Abnormal										X		X
ADA metabolite analysis (dATP) and ADA activity	Plasma deoxyadenosine level ADA (rbc) level dATP level	X		X	X	X	X	X	X	X	X	X	X
RCL (blood)	Reported as detectable / not detectable	X				X****	X****		X		X****		X

Assessment	Result to be reported on the CRF	PreGT	Treatment	1	1.5	3	6	9	12	18	24	30	36
Bone marrow aspirate for vector copy number qPCR and integration analysis (optional)	Morphology (Normal/ Abnormal) Vector copy number in BM-MNC Integration analysis (Normal / Abnormal)								X		X		X
Immunology save serum & SCLN (stored cells)	Serum collection date SCLN s collection date	X					X		X	X	X	X	X
Reporting of Adverse Event			X	X	X	X	X	X	X	X	X	X	X
Reporting of Concomitant Treatment^^			X	X	X	X	X	X	X	X	X	X	X

^Lab results to be entered into eCRF for these time points should be the ones closer to the consent date/ visit date
^^ Concomitant reporting: In case of any change in administering a concomitant medication i.e. dose, frequency etc. the previous medication should be stopped where the new medication has started
** Assay to be performed only if patient > 12 years of age*
*** Assay can only be performed if the lymphocyte count > 0.5 x 10^9/L*
 # Assay to be carried out until evidence of T cell recovery
**** Pre-gene therapy assay should be carried out only if not already done at diagnosis*
*****Sample are stored but analysed retrospectively only in case sample at 1 year visit or 3 year visit are positive*

PHA - phytohaemagglutinin stimulation, **Ig** – immunoglobulin, **TCR** - T cell receptor, **RCL** - replication competent lentivirus, **TREC** - TCR excision circles, **PCR** - polymerase chain reaction, **SCLN** - save cells in liquid nitrogen

4 Study Objectives

4.1 Primary Objectives

The primary objective is to assess the safety and efficacy of EFS-ADA LV mediated gene therapy, the Investigational Medicinal Product (IMP) for treatment of ADA-SCID patients. This will be achieved via the following specific objectives:

Efficacy

- To assess overall survival and event-free survival at 1 year for subjects treated with IMP and compare with patients treated with allogeneic HSCT
- To assess at each visit engraftment success and resulting immunological and metabolic effects in IMP treated subjects using:
  - Vector copy numbers in peripheral blood leukocytes
  - Cellular and humoral immune system recovery
  - ADA enzyme activity and reduction in dATP in peripheral blood cells

Safety

- To assess clinical, haematological and immunological progress of subjects
- To assess vector integration sites and clonal proliferation. This analysis will be performed at a specialised laboratory in Germany and the data will be reviewed and interpreted by the Principal Investigator (PI) and the study team

4.2 Secondary Objectives

The secondary objectives are to:

- Compare overall survival and event-free survival at 2 and 3 years between patients treated with IMP and patients treated with allogeneic HSCT
- Determine the percentage of IMP treated patients requiring immunoglobulin replacement therapy at all evaluation points from 18 months onwards
- Compare frequency of infections, and growth of pathogenic microorganisms over 3 years
- Evaluate the longitudinal clinical effect in terms of improved immunity
- Evaluate tolerability of conditioning regimen. These data will be reviewed and interpreted by the PI and the study team
- Evaluate feasibility of the transduction procedure. These data will be reviewed and interpreted by the PI and the study team.

5 Endpoints

5.1 Primary Endpoints

Primary endpoints for this study are:

- Overall survival at 1 year for patients treated with IMP.
- Event-free survival at 1 year for patients treated with IMP.

- Vector copy number in the cells at each study visit.
- Changes in T cell number, function and circulating immunoglobulin levels at each study visit.
- dATP and ADA enzyme activity in erythrocytes at each study visit.

The following endpoints will be analysed at a specialist laboratory in Germany and the results will be reviewed and interpreted by the PI and the study team, so these will not be included in this Statistical Analysis Plan (SAP).

- Frequency of vector integration into known proto oncogenes.
- Frequency of clonal expansion associated with vector integration near proto oncogene.

5.2 Secondary Endpoints

- Overall survival and event-free survival at 2 years and 3 years
- Frequency of infections (evaluated at 1, 2 and 3 years after treatment)
- TRECS after 2 years post-gene therapy
- Requirement for immunoglobulin replacement therapy (IgRT) and serum immunoglobulin levels for IgA, IgG and IgM.
- Feasibility of the transduction procedure assessed by availability of greater than 0.5 x 10^6 CD34+ cells/kg after transduction; undetectable RCL (determined retrospectively); and CD45+ cell viability after transduction equal to or greater than 50%, in accordance with the final product release criteria. These data will be reviewed and interpreted by the PI and the study team and so will not be included in this SAP.

5.3 Additional Endpoints

Additional endpoints for this study are:

- T cell Vβ panel
- Phytohaemagglutinin (PHA) stimulation result
- CD3 stimulation
- Vector integration analysis result
- Bone marrow aspirate (if performed)
  - Morphology (Normal/ Abnormal).
  - Integration (Normal/ Abnormal).
  - Vector copy number in BM-MNC.
- Tetanus vaccination response

6 Timing of Analyses

The statistical analyses of data from this study will be performed in four stages:

Interim look:

- Performed after all on-study subjects have completed 6 months of follow-up post infusion
- Descriptive statistics for all available data

- Comparisons between IMP treated subjects and HSCT control cohorts of demographics and overall survival

Primary analysis:

- Performed after all on-study subjects have completed 12 months of follow-up post infusion
- Descriptive statistics for all available data
- Comparison to HSCT control cohorts on overall and event-free survival at 12 months (primary efficacy endpoints), use of immunoglobulin replacement therapy (IgRT) and infection rate (key secondary endpoints).

Long-term follow-up analysis:

- Performed after all on-study subjects have completed 2 years of follow-up post infusion
- Descriptive statistics for all available data
- Comparison to HSCT control cohorts on overall and event-free survival at 2 years, use of immunoglobulin replacement therapy (IgRT) and infection rate (key secondary endpoints).

End of study analysis:

- Performed after all on-study subjects have completed the study (3 years of follow-up post infusion)
- Descriptive statistics for the complete study cohort
- Comparison to HSCT control cohorts on overall and event-free survival at 3 years, use of immunoglobulin replacement therapy (IgRT) and infection rate (key secondary endpoints).

7 Analysis Populations

7.1 Efficacy Population

IMP Treated Subjects

The study protocol recruited 10 subjects with a diagnosis of ADA-SCID to be treated with IMP at GOSH. These subjects will be known as the "on-study" subjects.

In addition, a group of subjects were treated under a Compassionate Use Programme (CUP under GOSH Special License) either because the study was not yet open and urgent treatment was needed, because they were outside of the inclusion/exclusion criteria or because they followed a different process (i.e., received the IMP in two infusions). These subjects followed the same study schedule as the on-study subjects.

The primary efficacy population for analysis will consist of the on-study IMP treated subjects. A secondary efficacy population will consist of all IMP treated subjects (on-study and CUP)

HSCT Control Patients

The primary efficacy population from the HSCT historical control cohort will consist of ADA-SCID patients without a medically eligible HLA-matched sibling/family donor and treated with HSCT at Great Ormond Street Hospital (GOSH) from the year 2000 onwards.

Secondary efficacy populations will comprise:

- ADA-SCID patients with matched related donors treated with HSCT at GOSH from the year 2000 onwards;
- the complete HSCT historical control cohort consisting of ADA-SCID patients with any type of donor treated with HSCT at GOSH from the year 2000 onwards.

7.2 Safety Population

The safety population will consist of all IMP treated subjects (on-study and CUP) and the complete HSCT historical control cohort consisting of ADA-SCID patients with any type of donor treated with HSCT at GOSH from the year 2000 onwards.

8 Statistical Methods

In general, continuous variables will be summarised using the mean, standard deviation, median, minimum, maximum and number of subjects. Categorical variables will be summarised using number of subjects and percentages.

For all summaries, the IMP treated subjects will be summarised in the groups of on-study only and on-study plus CUP subjects. The HSCT controls will be summarised in the groups of those without a matched related donor, those with a matched related donor and all subjects.

8.1 Subject Disposition

8.1.1 Summary of Populations

Number (%) of subjects enrolled (signed informed consent), treated, and in efficacy and safety populations will be summarised for the IMP treated and HSCT control groups. The denominator for the percentages will be the number of subjects enrolled.

Should any subjects be excluded from any of the analysis populations, a listing will be produced for these subjects noting the reason for exclusion.

8.1.2 Summary of Withdrawals

Number (%) of subjects who completed the study period will be summarised along with the reason for study withdrawal. The denominator for the percentages will be the number of subjects treated.

At the interim look, the primary analysis and the long-term follow-up analysis, the number (%) completing the designated follow-up period, and ongoing in the study will also be summarized.

Should any subjects have withdrawn from the study, a listing will be produced for these subjects noting the reason for study withdrawal, and the time since infusion at withdrawal.

8.2 Demography and Baseline Characteristics

Demography and baseline characteristics will be summarised for the safety population.

8.2.1 Demography

Age in months for the IMP treated on-study subjects will be calculated as:

12*(date informed consent signed – date of birth)/365.25.

Age in months for the IMP treated CUP subjects and the HSCT controls will be captured on the eCRF.

Age in months will be summarized for the IMP treated and HSCT control groups. Sex, ethnicity, location, weight and height at screening will be summarised for the IMP treated on-study group.

8.2.2 ADA Deficiency Diagnosis

Age at diagnosis in months will be calculated for the IMP treated subjects (on-study and CUP) as:

12*(date of diagnosis – date of birth)/365.25.

For the HSCT control groups, the age at diagnosis will be recorded on the eCRF.

Time since diagnosis in months will be calculated as:

12*(date of transplant - date of diagnosis)/365.25,

or, for HSCT control subjects for whom the dates were not recorded, as:

age in months at transplant - age in months at diagnosis.

Age at diagnosis and time since diagnosis will be summarized using descriptive statistics for the IMP treated subjects and HSCT control groups. In addition, the number (%) of subjects with each method of diagnosis (DNA sequencing/ confirmed absence of <3% enzymatic activity/ unknown) will be summarized. For DNA sequencing, the details of mutations, exons and polymorphisms will be listed.

8.2.3 Disease Treatment History

Number (%) of IMP treated subjects who previously received PEG-ADA treatment, receiving immunoglobulin replacement therapy, receiving infection prophylaxis, any previous BM or PBSC transplant (with the outcome) will be summarized.

8.2.4 Medical History

The number (%) of subjects reporting medical history in each category will be summarised for the IMP treated and the HSCT control groups.

8.2.5 Infection at Screening

The number (%) of IMP treated subjects reporting active infections at screening will be summarised overall and by localisation/event type.

8.2.6 Coagulation Tests

Coagulation tests (partial thromboplastin time, APT, thrombin time and fibrinogen) will be evaluated at screening for the IMP treated subjects and summarised using descriptive statistics.

8.2.7 Glomerular Filtration Rate (GFR) at screening

Distribution volume, half-life, glomerular filtration rate and corrected glomerular filtration rate will be summarised for the IMP treated subjects using descriptive statistics. In addition, the number (%) of subjects with a distribution volume outside the normal range that was considered to be clinically non-significant or clinically significant will be summarized.

8.2.8 Virology Tests

The number (%) of IMP treated subjects with positive results for CMV, EBV, Adenovirus, HIV1, HIV2, VZV, Hep A, Hep B, HSV, Hep C, Syphilis, HTLV1, HTLV2, Toxoplasma will be summarised.

8.2.9 Cardiopulmonary Tests at Screening

A chest x-ray, ECG, Echocardiogram and lung function test will be carried out at screening. The results of each test will be categorized as normal/ abnormal, not clinically significant/ abnormal, clinically significant. The number (%) of IMP treated subjects with evaluations falling into each category will be summarised for each test. Should any subject have an abnormal evaluation with the clinical significance assessment missing, this will be categorized as “abnormal unknown clinical significance” and this category will be added to the summary.

In addition, details of any clinically significant findings will be listed.

8.2.10 General Tests at Screening

Dental, audiology and maternal engraftment studies tests will be carried out at screening. The results of each test will be categorized as normal/ abnormal, not clinically significant/ abnormal, clinically significant. The number (%) of IMP treated subjects with evaluations falling into each category will be summarised for each test. Should any subject have an abnormal evaluation with the clinical significance assessment missing, this will be categorized as “abnormal unknown clinical significance” and this category will be added to the summary.

In addition, details of any clinically significant findings will be listed.

8.2.11 Harvest for Gene Therapy

The number (%) of IMP treated subjects with each blood type and with stem cells from each source (leukapheresis/bone marrow) will be summarised.

For subjects with a leukapheresis harvest, the dose of G-CSF administered on Days 1-6, the details of any plerixafor administration and the $CD34^+$ cell counts in blood will be listed.

For subjects with a leukapheresis harvest on multiple days, the total number of cells will be the sum across the days. The number of cells stored for the gene therapy will be calculated as the total cell count – number of back up cells. Body weight at harvest, total number of nucleated cells, total $CD34^+$ cell count and $CD34^+$ cell count excluding back-up cells will be summarised using descriptive statistics.

8.2.12 Back-up Harvest

The number (%) of IMP treated subjects for whom $\geq 3x10^6$ $CD34^+$ cells/kg have been stored as back-up will be summarised along with the $CD34^+$ count. For those that have a back-up harvest, the number (%) with stem cells from each source (leukapheresis/bone marrow) will be summarized using the number with a back-up harvest as denominator for the percentage. For those without a back-up harvest, the number (%) with each reason for not performing the harvest will be summarized using the number without a back-up harvest as denominator for the percentage.

8.2.13 Myeloablative Conditioning

The number (%) of IMP treated subjects for whom conditioning was done will be summarised along with the body weight, height, BSA, busulfan dose and busulfan AUC.

8.2.14 IMP Administration

Age at transplant in months will be calculated for the IMP treated subjects (on-study and CUP) as:

12*(date of transplant – date of birth)/365.25.

The duration of IMP infusion (in minutes) will be calculated as (end time – start time)/60.

The number (%) of subjects who received the IMP infusion and for whom the entire content of the bag was infused will be summarised along with the age at transplant, the duration of infusion, $CD34^+$ cell dose, cell viability and volume.

8.2.15 Transplant Details for the HSCT Controls

For the HSCT control groups, the age in months at transplant will be recorded on the eCRF.

Age (months) at transplant, number of $CD34^+$ cells/kg transplanted and busulfan dose, if appropriate, will be summarized for each HSCT control group using descriptive statistics.

Number (%) of subjects with each type of transplant cells, transplant donor type, conditioning type, receiving enzyme replacement therapy (ERT) prior to the transplant, receiving ERT after the transplant, receiving IgRT prior to the transplant, receiving IgRT after the transplant and receiving gene therapy after the transplant will be summarized for each HSCT control group.

8.2.16 Exposure

Duration of follow-up in months will be calculated for the IMP treated subjects as:

12*(date of final evaluation – date of infusion + 1)/365.25.

For the HSCT controls, the duration of follow-up will be recorded on the eCRF.

Duration of follow-up will be summarized for the IMP treated and the HSCT control groups using descriptive statistics. In addition, the total subject-years of follow-up in each treatment group will be presented.

8.3 Efficacy Analyses

The primary comparison for all efficacy analyses will be between the IMP treated on-study subjects and the allogeneic HSCT controls treated at GOSH without a matched related donor. Secondary comparisons will be between:

- the IMP treated on-study subjects and the HSCT controls treated at GOSH with a matched related donor;
- the IMP treated on-study subjects and the total group of HSCT controls;
- all IMP treated subjects (on-study and CUP) and the HSCT controls treated at GOSH without a matched related donor;
- all IMP treated subjects (on-study and CUP) and the HSCT controls treated at GOSH with a matched related donor;
- all IMP treated subjects (on-study and CUP) and the total group of HSCT controls.

8.3.1 Primary Efficacy Endpoints

8.3.1.1 Overall Survival at 12 Months

Overall survival will be evaluated using the time in months from transplant to either the subject's death or their last evaluation calculated as:

12*(date of death/last evaluation – date of transplant)/365.25,

or, for HSCT control subjects for whom the dates were not recorded, as:

age in months at death/end of follow-up - age in months at transplant.

The number (%) of subjects who were still alive at 12 months post-transplant will be summarised. For the purpose of this analysis, any subject who withdrew from the study prior

to 12 months, who was known to be alive at the last evaluation, will have a status of "unknown". The proportion and exact 95% confidence interval (CI) for survivors at 12 months post-transplant will be calculated for each of the IMP treated and HSCT control groups along with the difference in proportions (with 95% CI) between the IMP treated and control groups, excluding those with unknown status.

Overall survival in each treatment group will also be represented using a Kaplan-Meier curve for time to death. For the purpose of this analysis, any subject who withdrew from the study prior to 12 months and was known to be alive at the last evaluation will be censored at the date of their last evaluation. If appropriate, the proportion alive at 12 months and the associated 95% CI will be estimated for each treatment group from the Kaplan-Meier curve along with the median survival time (and interquartile range), if they can be estimated from the data.

If there is at least one event in each group, the log-rank test will be used to compare the difference in survival curves between the IMP treated and HSCT control groups.

8.3.1.2 Event-free Survival at 12 Months

Event-free survival will be evaluated using the time in months from transplant to either the first event or their last evaluation calculated as:

12*(date of event/last evaluation – date of transplant)/365.25,

or, for HSCT control subjects for whom the dates were not recorded, as:

age in months at death/end of follow-up - age in months at transplant.

For the purpose of this analysis, the events of interest are:

- death;
- reinstitution of PEG-ADA ERT;
- requirement for a second transplant.

Event-free survival will be summarized in the same way as overall survival described in Section 8.3.1.1.

In addition, if there are any events (other than death), the time to each event will be summarized in a similar manner to the overall event-free survival.

8.3.1.3 Engraftment

Engraftment of transduced cells will be assessed using vector gene marking in granulocytes, PBMC, CD3, CD34, CD19 and myeloid progenitor cells. Any assessments in which the result indicates that cells were not detected, will be assigned a value of 0.

The vector copy numbers in each type of cell will be summarised for IMP treated subjects by visit using descriptive statistics. In addition, for each cell type, plots of the median and range for copy numbers over time on a logarithmic scale will be produced along with individual subject plots.

8.3.1.4 Cellular and Humoral Immune System Recovery

Immune reconstitution will be assessed using lymphocyte subsets. Absolute numbers and percentages of $CD3^+$, $CD4^+$, $CD8^+$ $CD4^+CD45RA^+CD27^+$ T-lymphocytes, $CD19^+$ B-lymphocytes and $CD16^+CD56^+$ NK cells will be measured.

For each of the lymphocyte subsets, the actual values and change from baseline will be summarized by visit for each of the IMP treated groups using descriptive statistics. The median value and range will be plotted by treatment group over time on a logarithmic scale and individual counts will be plotted along with the age-dependent normal ranges provided by the study site.

In addition, statistical testing of the change from baseline to 6 and 12 months will be performed for the IMP treated subjects. If the difference is normally distributed, a paired t-test will be used. Otherwise, the Wilcoxon signed rank test will be used.

The trend over time in the IMP treated subjects will be examined for each count separately, using mixed models for repeated measures (MMRM) with visit and baseline count as fixed effects, and subject as a random effect, utilizing the compound-symmetry covariance structure. If counts are approximately normally distributed they will be analysed on the original scale. Otherwise, if log transformation can normalize the data, statistical analysis will be performed on log-transformed data. If the data are still not normal, the data will be ranked in ascending order of magnitude and the analysis will be performed on the ranks.

8.3.1.5 RBC ADA Enzyme Activity and Reduction in dATP

RBC ADA enzyme activity, dATP and plasma deoxyadenosine will be summarized by visit for each of the IMP treated groups using descriptive statistics. The median value and range will be plotted by treatment group over time on a logarithmic scale. In addition, individual subject plots will be created.

Changes from baseline in each of the parameters will be calculated with baseline being the pre-treatment evaluation. The change from baseline will be summarized by visit for each of the IMP treated and HSCT control groups (data permitting) using descriptive statistics and 95% confidence intervals.

8.3.2 Secondary Efficacy Endpoints

8.3.2.1 Overall and Event-free Survival at 2 and 3 Years

Overall and event-free survival at 2 and 3 years will be summarized in the same way as for the analyses at 12 months described in Sections 8.3.1.1 and 8.3.1.2.

8.3.2.2 Use of Immunoglobulin Replacement Therapy

The time to cessation of IgRT will be calculated (in months) as:

12*(stopping date of IgRT – date of transplant) /365.25,

or, for HSCT control subjects for whom the dates were not recorded, as:

age in months when IgRT stopped – age in months at transplant.

Time to cessation of IgRT will be summarised for each of the IMP treated and HSCT control groups using descriptive statistics and, if appropriate, estimated from a Kaplan-Meier curve in which subjects who are still receiving IgRT will be censored at the time of their last visit.

The number (%) of subjects who have stopped treatment with IgRT at 12, 18, 24, 30 and 36 months without restarting at any subsequent time point will be summarized for each of the IMP treated and HSCT control groups (data permitting) along with exact 95% CIs.

In addition, serum immunoglobulin levels (IgG, IgA and IgM) will be measured at each visit for the IMP treated subjects. Levels of IgG, IgA and IgM and changes from baseline will be summarised by visit using descriptive statistics. In addition, plots of the median value and range over time on a logarithmic scale will be produced for each parameter along with individual plots for each subject which will include age-dependent normal ranges provided by the study site.

8.3.2.3 Rate of Severe Infections

Infections will be recorded as adverse events. The infections of interest in this study are severe infections or opportunistic infectious episodes, defined as infections requiring hospitalization or prolonging hospitalization and/or documented infections by opportunistic pathogens (e.g. interstitial pneumonia, intractable diarrhoea).

The number (%) of subjects reporting severe infections or opportunistic infectious episodes along with the number of events reported will be summarized for each of the IMP treated and HSCT control groups (data permitting). In addition, the infection rate will be calculated as the number of infections/the total follow-up time. The rate will be calculated both within each treatment group and for each individual subject. The subject infection rate will be summarized by treatment group using descriptive statistics. In addition to the rate for the entire follow-up period, the rate will be calculated for both the first 12 months following transplant and for the second and third years.

8.3.2.4 T Cell Receptor Excision Circles (TREC) in the Peripheral Blood

TREC levels on T cells and mononuclear cells will be summarised for each group of IMP treated subjects at each visit using descriptive statistics. Plots of the median and range over time on a logarithmic scale will be produced. In addition, individual subject plots of TREC levels over time will be produced.

8.3.3 Additional Efficacy Endpoints

8.3.3.1 Use of PEG-ADA Enzyme Replacement Therapy

The time to withdrawal of PEG-ADA ERT will be calculated (in months) as:

12*(stop date of PEG-ADA ERT – date of transplant) /365.25,

or, for HSCT control subjects for whom the dates were not recorded, as:

age in months when PEG-ADA stopped – age in months at transplant.

Time to withdrawal of PEG-ADA ERT will be summarised for each of the IMP treated and HSCT control groups using descriptive statistics and, if appropriate, estimated from a Kaplan-Meier curve in which subjects who are still receiving PEG-ADA ERT will be censored at the time of their last visit.

8.3.3.2 T Cell Vβ Panel

Results of the T cell Vβ panel spectratyping will be reported as normal or abnormal. The number (%) of subjects with normal/abnormal results will be summarized by visit for each of the IMP treated groups along with the number (%) with an abnormal result at any post-infusion visit.

8.3.3.3 PHA Stimulation

PHA stimulation results will be categorised as normal proliferation, impaired proliferation, absence of proliferation or other. The number (%) of subjects falling into each category will be summarized by visit for each of the IMP treated groups.

The PHA stimulation index will be calculated as the stimulated PHA/unstimulated PHA and will be summarized at each visit for each of the IMP treated groups. In addition, the median and range will be plotted over time on a logarithmic scale.

8.3.3.4 CD3 Stimulation

CD3 stimulation results will be reported as normal or abnormal. The number (%) of subjects with normal/abnormal results will be summarized by visit for each of the IMP treated groups along with the number (%) with an abnormal result at any post-infusion visit.

8.3.3.5 Vector Integration Analysis

The number (%) of subjects with one integration site representing > 30% of total integration will be summarized by visit for each of the IMP treated groups. The number (%) of subjects for whom one integration site contributed > 30% of total integration at 2 or more time points will be summarized for each of the IMP treated groups. In addition, the total number of integration sites will be summarized by visit for each of the IMP treated groups.

8.3.3.6 Bone Marrow Aspirate

A bone marrow aspirate may be performed for vector copy number, qPCR and integration analysis. Morphology and integration analysis results will be reported as normal or abnormal. The number (%) of subjects with normal/abnormal results for each of the tests will be summarized by visit for each of the IMP treated groups. In addition, the vector copy number in BM-MNC will be summarised using descriptive statistics.

8.3.3.7 Response to Tetanus Vaccination

The response to the tetanus vaccination will be reported as normal or abnormal. The number (%) of subjects with normal/abnormal results will be summarised for each of the IMP treated groups.

8.3.4 Sensitivity Analyses

Protocol violations in this study will be reviewed prior to database lock in order to determine whether any major violations have occurred that could have influenced the outcome of the study. In the event that such violations have occurred, sensitivity analyses will be carried out in order to assess the impact of these violations on the efficacy results.

Sensitivity analyses will be carried out for:

- Overall and event free survival (descriptive statistics and Kaplan-Meier analyses)
- Lymphocyte subsets (descriptive statistics and plots)

- Measures of engraftment (descriptive statistics and plots)

8.4 Safety Analyses

8.4.1 Adverse Events

All adverse events will be coded using MedDRA version 20.0 or higher

8.4.1.1 Definition of Treatment-emergent Adverse Events

Adverse events (AEs) will be summarised on the basis of treatment emergence. An adverse event is considered to be treatment emergent if it occurred on or after the date and time of the start of the IMP infusion. If the start time of the event is not present, any event starting on the same day as the infusion will be considered to be treatment emergent. If the start date of the adverse event is partial or missing, it will be considered to be treatment emergent if:

- the day is missing, the month and year are present and are the same as or after the month and year of the infusion and the end date is missing or is not before the date of infusion,
- the day and month are missing, the year is present and is the same as or after the year of the infusion and the end date is missing or is not before the date of infusion,
- the entire date is missing and the end date is missing or is not before the date of infusion.

8.4.1.2 Adverse Events

Treatment emergent adverse events will be summarised for each of the IMP treated groups in an overall summary presenting the number (%) of subjects with:

- any AE,
- moderate or severe AE,
- severe AE,
- treatment related AE (possibly/probably/definitely/not assessable/missing),
- AE leading to study withdrawal,
- any serious adverse event (SAE),
- treatment related SAE,
- SAE leading to death.

Incidence and frequency of treatment emergent AEs will be summarised for each of the IMP treated groups by system organ class (SOC) and preferred term (PT):

- overall,
- by maximum severity,
- by maximum relationship to treatment,
- treatment related AEs,
- AEs leading to study withdrawal (if sufficient events).

In addition, a summary table for the most frequently reported treatment emergent adverse events (those reported in 2 or more subjects) will be presented by PT in descending order of incidence and frequency.

In each table, if the same SOC or PT is reported on multiple occasions for a single subject, it will only be included once in the summary. In the table by maximum severity, only the most severe occurrence for each SOC and PT for each subject will be included in the summary. In the table by maximum relationship, only the most related occurrence for each SOC and PT for each subject will be included in the summary.

Any adverse events that are not treatment emergent (occurred prior to the infusion) will be listed.

8.4.1.3 Serious Adverse Events and Death

Incidence and frequency of treatment emergent SAEs will be summarised for each of the IMP treated and HSCT control groups (data permitting) by system organ class (SOC) and preferred term (PT):

- overall,
- by maximum severity,
- by maximum relationship to treatment,
- treatment related SAEs.

In each table, if the same SOC or PT is reported on multiple occasions for a single subject, it will only be included once in the summary. In the table by maximum severity, only the most severe occurrence for each SOC and PT for each subject will be included in the summary. In the table by maximum relationship, only the most related occurrence for each SOC and PT for each subject will be included in the summary.

Any SAEs that are not treatment emergent (occurred prior to the infusion) will be listed.

If there are any deaths, the details will be listed.

8.4.2 Concomitant Medications

Concomitant medications taken during the study will be listed.

8.4.3 RCL Testing

Number (%) of subjects testing positive for RCL will be summarised for each of the IMP treated groups by visit and at any time.

8.4.4 Laboratory Evaluations

8.4.4.1 Haematology

Haematology parameters (red blood cell (RBC) count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), neutrophils, lymphocytes,

monocytes, eosinophils, basophils and platelet count) will be evaluated over time for the IMP treated subjects. The baseline evaluation for each parameter will be the final evaluation prior to the IMP infusion.

For each parameter, the actual value and change from baseline will be summarised at each visit using descriptive statistics for each of the IMP treated groups.

Haematology evaluations will be flagged against the normal range as low/normal/high. For each parameter, the number (%) of subjects with evaluations that were low/normal/high relative to the normal range will be summarised by visit. In addition, shift tables will be constructed comparing the flags at the baseline evaluation with those at each post-infusion evaluation and the minimum and maximum evaluation post-infusion. Out of range values will be assessed for their clinical significance. The number (%) of subjects with any clinically significant abnormal values will be summarized by parameter at each visit and at any time post-infusion.

8.4.4.2 Biochemistry

Biochemistry parameters (sodium, glucose, chloride, potassium, urea, creatinine, calcium, magnesium, phosphates, albumin, alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), bilirubin, total protein) will be evaluated over time for the IMP treated subjects. The baseline evaluation for each parameter will be the final evaluation prior to the IMP infusion.

For each parameter, the actual value and change from baseline will be summarised for each IMP treated group at each visit using descriptive statistics.

Biochemistry evaluations will be flagged against the normal range as low/normal/high. For each parameter, the number (%) of subjects with evaluations that were low/normal/high relative to the normal range will be summarised by visit. In addition, shift tables will be constructed comparing the flags at the baseline evaluation with those at each post-infusion evaluation and with the minimum and maximum evaluation post-infusion. Out of range values will be assessed for their clinical significance. The number (%) of subjects with clinically significant abnormal values will be summarized by parameter at each visit and at any time post-infusion.

8.4.5 Vital Signs and Weight

Vital signs (temperature, pulse rate, respiratory rate, oxygen saturation) and weight are measured over time for the IMP treated subjects.

The baseline evaluation for each of the vital signs and weight will be the final evaluation prior to the IMP infusion.

For each parameter, the actual value and change from baseline will be summarised for each of the IMP treated groups at each visit using descriptive statistics.

Each subject's vital signs be categorized as normal, abnormal not clinically significant or abnormal clinically significant at each visit. The number (%) of subjects with clinically significant evaluations will be summarised for each parameter by visit and at any time post-infusion. In addition, the number (%) of subjects requiring additional O_2 will be summarised by visit.

8.4.6 Physical Examination

Physical examinations will be performed over time for the IMP treated subjects. The number (%) of subjects with abnormal evaluations will be summarized by body system for each of the IMP treated groups at each visit. An overall summary will also be produced for each body system showing the number (%) of subjects with normal evaluations at screening who had one or more abnormal evaluations post-treatment.

9 Analysis Software

All summaries and analyses will be carried out using SAS version 9.4 or higher.

10 Changes in Statistical Analysis Plan

10.1 Changes in Version 10.0

Since Statistical Analysis Plan version 9.0 was signed off on 14th March, 2017, Version 17.0 of the protocol has been signed off and changes have been made to the eCRF, so a number of modifications were needed to this SAP in order to account for the changes in the protocol and eCRF.

In addition to these changes, the document has been restructured in order to improve the flow and the understandability. In particular, the description of any derivations of the endpoints has been added to the statistical methods section instead of being with the list of endpoints. Also, some of the details relating to programming specifications have been removed and will be added to the programming documentation.

Changes of note are:

- Study objectives and endpoints have been reworded to match protocol version 17.0.
- The age at entry and transplant will be expressed in months rather than years as this is more appropriate for the subjects enrolled.
- The calculation of durations in months has been changed from days/30 to 12*days/365.25 to be consistent with calculations of age.
- Inclusion/exclusion criteria will no longer be summarized, but will be listed.
- The plots of parameters over time will no longer present the mean +/- 2SE, but the median and range as this was more appropriate for the parameters being presented.

- The eCRFs for the vector integration and PHA stimulation have been changed, so the summaries have been modified to take account of the change in the data being collected.
- The infection summaries have been reworded to match the definition of severe infections from the protocol.
- Description of possible sensitivity analyses has been added.
- Additional adverse event summaries have been added and it has been explicitly stated that all summaries will include only treatment emergent adverse events.
- Summaries of laboratory evaluations using descriptive statistics have been added.
- Plots of vital signs over time have been removed as they were not considered to be useful.

10.2 Changes in Version 11.0

Since version 10.0 of the Statistical Analysis Plan was signed off, the sponsor has received regulatory approval to include further HSCT control data from Duke University, so this SAP has been updated to include these data.

The following additional changes have been made:

- Details added to clarify the calculation of time to event for subjects in the HSCT control group who do not have the dates of events recorded.
- Tables of reference ranges have been deleted to allow the most up to date ranges received from the study site to be used in the analyses.
- The summary of coagulation data has been removed from the safety section as these data are only collected at screening.
- The MedDRA version to be used has been updated to 20.0.

10.3 Changes in Version 12.0

Since version 11.0 of the Statistical Analysis Plan was signed off, the sponsor has made the decision to restrict the HSCT control data to be only those from subjects treated at GOSH, so this SAP has been updated to take account of this change.