1 April 2016
EMA/CHMP/272303/2016 Rev 1.
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Strimvelis

Common name: autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence

Procedure No. EMEA/H/C/003854/0000

Note

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

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List of abbreviations

ADA Adenosine deaminase

ADA-SCID Adenosine deaminase severe combined immunodeficiency

ADR Adverse drug reaction

AE Adverse event

BMT Bone marrow transplantation

CAT Committee for Advanced Therapies

cDNA Complementary deoxyribonucleic acid

CGD Chronic granulomatous disease

CHMP Committee for Medicinal Products for Human Use

CNS Central nervous system

CPP Critical process parameters

CQA Critical Quality Attributes

CSR Clinical study report

CUP Compassionate use program

CVC Central venous catheter

dATP Deoxyadenosine triphosphate

dAXP Deoxyadenosine nucleotides

deaminase

EBMT European Group for Blood and Marrow Transplantation

EPC End of Productions cells

ERT Enzyme replacement therapy

ESID European Society for Immunodeficiencies

EU European Union

FBS Foetal bovine serum

FMEA Failure Mode Effect Analysis

Fondazione Centro San Raffaele del Monte Tabor)

GCP Good clinical practice

GSK GlaxoSmithKline

GTV Gene Therapy Vector

GvHD Graft versus host disease

HCP Host Cell Protein

HLA Human leukocyte antigen

HAS Human Serum Albumin

HSC Hematopoietic stem cells

HSR-TIGET Hospital San Raffaele Telethon Institute for Gene

Instituto Scientifico San Raffaele

IPC In-process control

IPS In-process specification

IV Intravenous

IVIG Intravenous immunoglobulin

JACIE Joint Accreditation Committee Evaluation program

kg Kilogram

L Liter

LTFU Long-term follow-up

MAA Marketing Authorisation Application

MCB Master Cell Bank

MDS Myelodysplastic syndrome

MedDRA Medical Dictionary for Regulatory Activities

MFD Matched family donor

mg Milligram

mL Milliliter

MLV Murine leukemia virus

Mmol Millimolar

MoMLV Moloney Murine Leukaemia Virus

MOI Multiplicity of infection

MSD Matched sibling donor

MUD Matched unrelated donor

nmol Nanomolar

NPP Named Patient Program

OMIM Online Mendelian Inheritance in Man

PEG-ADA Polyethylene-glycol-modified bovine adenosine

PPCB Post-production cell bank

PPQ Process performance qualification

RBC Red blood cell

RCR Replication competent recombinant retrovirus

RCR Replication competent retrovirus

RIS Retroviral insertion site

RMP Risk Management Plan

SAE Serious adverse event

SCE Summary of Clinical Efficacy

SCID Severe combined immunodeficiency

SCS Summary of Clinical Safety

SCT Stem cell transplant

SOC System Organ Class

SPC Summary of Product Characteristics

Therapy; Ospedale San Raffaele s.r.l. (formerly

TREC T cell receptor excision circles

TSE transmissible spongioform encephalitis

US United States

UTI Urinary tract infection

VCN Vector copy number

VCN(o) Vector copy number overall, calculated as average based on total cells in the sample

VCN/TC Vector copy number per transduced cell, calculated as an average based VCN and the % transduction efficiency

WAS Wiskott-Aldrich Syndrome

WBC White blood cells

X-SCID X-linked SCID

1. Background information on the procedure

1.1. Submission of the dossier

The applicant GlaxoSmithKline Trading Services submitted on 1 May 2015 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Strimvelis, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 19 September 2013.

Strimvelis was designated as an orphan medicinal product EU/3/05/313 on 26/08/2005 in the following indication: Treatment of severe combined immunodeficiency (SCID) due to adenosine deaminase (ADA) deficiency.

The applicant applied for the following indication: Strimvelis is indicated for the treatment of patients with severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID), for whom no suitable human leukocyte antigen (HLA)-matched related stem cell donor is available. The approved indication is the same as the indication applied for.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Strimvelis as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website: ema.europa.eu/Find medicine/Rare disease designations.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that Autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0190/2014 on the agreement of a paediatric investigation plan (PIP). At the time of submission of the application, the PIP P/0190/2014 was completed.

The PDCO issued an opinion on compliance for the PIP P/0190/2014.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance Autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence contained in the above medicinal product to be considered as a new active substance in itself, as the

applicant claims that it is not a constituent of a product previously authorised within the Union.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 21/06/2007, 25/09/2008, 23/06/2011, 20/02/2014 and 11/03/2014. The Protocol Assistance pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status

Strimvelis was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Elaine French Co-Rapporteur: Sol Ruiz

CHMP Coordinator (Rapporteur): Robert Hemmings

CHMP Coordinator (Co-Rapporteur): Concepcion Prieto Yerro

- The application was received by the EMA on 1 May 2015.
- The procedure started on 28 May 2015.
- The CAT agreed to consult the national Notified Bodies on the Environmental Risk Assessment of the GMO as the ATMP is a gene therapy product. The consultation procedure closed on 25 February 2016.
- The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 17 August 2015. The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 14 August 2015.
- The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 10 September 2015.
- During the meetings on 18 and 24 September 2015, the CAT and CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 25 September 2015.
- The applicant submitted the responses to the CAT and CHMP consolidated List of Questions on 21 December 2015.
- A GMP inspection was requested by the CHMP and the outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CAT and CHMP members on 01 February 2016.
- The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 11 February 2016.
- During the CAT and CHMP meetings on 19 and 25 February 2016, the CAT and CHMP agreed on a List of Outstanding Issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CAT and CHMP List of Outstanding Issues on 26 February 2016.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CAT and CHMP members on 11 March 2016.
- The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 17 March 2016.
- During the meeting on 23 March 2016, the CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive draft opinion for granting a Marketing Authorisation to Strimvelis.
- During the meeting on 1 April 2016, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Strimvelis.

2. Scientific discussion

2.1. Introduction

Severe combined immunodeficiency (SCID) due to adenosine deaminase (ADA) deficiency is a fatal autosomal recessive monogenic inherited immune disorder. The main features of ADA-SCID are profound lymphopenia, impaired differentiation and function of T cells, B cells, and natural killer (NK) cells; recurrent infections; and failure to thrive. Non-immunological abnormalities may also occur as a consequence of the systemic metabolic defect and include hepatic, lung, and renal disease, lymphoma, often associated with cells bearing Epstein-Barr virus (EBV) genomes, skeletal alterations, and neurological deficits affecting motor function and hearing, and cognitive/behavioural deficits, indicating that ADA-SCID is more complex than other forms of SCID. ADA-SCID is ultra-rare, with an overall incidence ranging from 0.22 to 0.68 per 100,000 live births, or less than 50 children per year in the United States (US) and European Union (EU) combined. The EMA have recognised the importance of supporting development of new treatments for Rare Diseases through its alliance with EURORDIS and through the FP7 and FP8 frameworks which support innovative research in non-infectious non-cancer diseases such as ADA-SCID. The majority of patients with ADA-SCID are diagnosed in the first year of life and rarely survive beyond 1 to 2 years unless immune function is restored.

Current Therapeutic Options for ADA-SCID

Bone Marrow Transplant: Bone marrow transplant, also known as stem cell transplant (SCT), is a potentially curative treatment for ADA-SCID but the effectiveness of the treatment is heavily dependent upon the degree of HLA matching between the donor and the patient. A recent analysis of outcomes in 106 children with ADA-SCID confirmed similar overall 6.5 year survival rates after transplant from matched sibling or matched family donor of 86% and 83%, respectively. However, the outcomes are less favourable in the majority of patients for whom matched related donors are not available. When patients are treated with cells from less well matched donors, the main risk is graft versus host disease (GvHD), a situation where the donor graft recognises the patient as foreign and mounts an immune response. The GvHD is usually managed with pre-transplant chemotherapy and potent immunosuppressive medicine, which on the other hand, increases the risk of infection. Patients for whom no suitable HLA matched related stem cell donor is available might represent a population suitable for Strimvelis treatment.

Enzyme Replacement Therapy: Enzyme replacement therapy (ERT) with polyethylene-glycol-modified bovine ADA (PEG-ADA), while not approved in Europe, is available in some Member States via compassionate use. Patients require ERT as either weekly or bi-weekly intramuscular injections in order to control disease symptoms. A retrospective study of the long-term effects of PEG-ADA treatment for 5 to 12 years found that, despite initial improvements, lymphocyte counts were below the lower limit of normal for all patients and progressively worsened over time. There was also a gradual reduction in thymic function and a decline in mitogenic proliferative responses over time, demonstrating reduced T cell function. It is thought that metabolic reconstitution in the thymus is incomplete, and this leads to the gradual loss of immune function. In published results of patients who received ERT between 1986 and 2008, the overall probability of 20-year-survival on ERT was estimated to be 78%; however, 18% of patients died due to causes related to immune deficiency. Half of the deaths on ERT occurred within the first 6 months of treatment and resulted from conditions present at diagnosis (refractory haemolytic anaemia, chronic pulmonary insufficiency, and lymphoproliferative disorders). Approximately 50 to 60% of children treated with PEG-ADA develop anti-ADA antibodies, and for 10% of the treated children (i.e., in approximately 20% of the children that develop antibodies) this leads to neutralization of ADA activity, requiring an increase in dose,

administration of corticosteroids, or cessation of therapy. Development of sustained autoimmune manifestations has been reported in 5% of patients receiving PEG-ADA, including 3 cases of fatal autoimmune haemolytic anaemia.

Unmet Medical Need in ADA-SCID: Based on the estimate of 50 patients per year born with ADA-SCID, 5 – 10 will be treatable with SCT from a HLA matched related donor. The remaining patients will receive SCT from a less well matched donor or ERT. The main problems associated with SCT from less well matched donors arise from the use of chemotherapeutic pre-conditioning and clinical management of GvHD with immunosuppressive drugs that further increase the risk of infection. The mortality following transplant arises mainly due to infection or management of GvHD (>50% sepsis, respiratory failure and pneumonitis, 15 % GvHD and 11% fungal infection). The 6.5-year survival rate for these patients ranges from 67% for patients treated with SCT from a matched unrelated donor to 29% for patients treated with SCT from a haploidentical donor. Enzyme replacement therapy with PEG-ADA is not approved for use in Europe, and although it is often available through compassionate use programmes, not all patients have access to it. Additionally, patients depend on continuous weekly or bi-weekly treatments, while immune reconstitution is variable, and long-term efficacy is limited. Hence, for those ADA-SCID patients for whom no suitable HLA matched related donor is available, there is a high unmet need for new treatment options that would provide long term corrective therapy with an improved probability of survival.

Strimvelis is a gene therapy product manufactured from the patient's own CD34+ cells, so called autologous cells. The use of the patient's own cells eliminates the risk of GvHD. The lack of GvHD risk means that patients are less likely to need immunosuppression and are therefore at less risk from infection which is the main cause of death after transplantation in ADA-SCID patients. Furthermore, gene therapy is not dependent upon a donor search, so it can be made available to any patient. Srimvelis is proposed to be a one off treatment, intended to provide life-long benefit. This compares favourably to ERT which requires frequent injections (weekly or bi-weekly) and regular monitoring of dATP metabolite levels and antibody formation against PEG-ADA.

2.2. Quality aspects

2.2.1. Introduction

Strimvelis is a gene therapy product which contains autologous genetically modified CD34+ cells. The product is manufactured from the patient's own CD34+ cells, which are transduced with retroviral vector GSK3336223 that encodes for the human enzyme adenosine deaminase (ADA) cDNA sequence.

The finished product is presented as dispersion for infusion containing 1 – 10 million cells/ml of autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence. The cells are formulated in 0.9% sodium chloride.

Strimvelis contains as other ingredients only sodium chloride.

Strimvelis is supplied in one or more sterile ethylene vinyl acetate (EVA) bag, 50 mL nominal fill, with a Luer spike interconnector closed with a Luer lock cap.

2.2.2. Active Substance

General information

The active substance consists of autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector GSK3336223 that encodes for the human ADA cDNA sequence.

The section on the active substance is separated into two parts on the gene therapy vector (GSK3336223) (Part 1) and on the transduced CD34+ cells (Part 2).

Part 1: Gene Therapy Vector (GSK3336223)

GSK3336223 is a replication deficient gamma-retroviral vector based on Moloney murine leukaemia virus (MoMLV) encoding the cDNA sequence for the human ADA.

Manufacture, characterisation and process controls

Manufacturing Process

The manufacture of the retroviral vector is performed at MolMed S.p.A., Via Olgettina, 58, 20132 Milano, Italy.

The vector production process consists of thawing and expansion of the producer cell line, incubation with harvest medium and harvest. This is followed by filtration and freezing. An extensive end-of-production (EPC) library is established from the producer cell line for the purposes of quality control.

The applicant has provided an adequate description of the vector manufacturing process. This includes a sufficiently detailed description of the manufacturing process steps, flow-charts and separate tables of process parameters (PPs), critical process parameters (CPPs), in-process controls (IPCs) and in-process specifications (IPSs), which give operating ranges, set points and acceptance limits. All CPPs, IPCs and IPS have been listed. The applicant has stated that deviations from CPP ranges and IPCs will be subject to investigation to determine the impact on CQAs.

The microbiological control strategy has been set out and is overall acceptable.

A sufficient description of the batch size and numbering system has been given.

Control of materials

GSK3336223 is based on the MoMLV virus with added safety features to prevent generation of replication competent retroviruses (RCRs). The applicant states that RCR has never been detected.

Sufficient description on the construction of the vector and production cell lines was given. A full list of the genetic elements and sequence of the provirus was provided.

The applicant has given a description of the production and analysis of the master cell bank (MCB), working cell bank (WCB) and Post-production cell bank (PPCB). From the transfer to MolMed onward, banks were laid down in accordance with GMP and only animal material with low risk of TSE was used. A full description of the process and materials used was provided.

Tests performed on the MCB include viability and recovery at thaw, consistency of growth profile, viral titre and transduction efficiency, RCRs, vector integration and integrity and sequencing.

Testing for adventitious agents was conducted in compliance with ICH Q5A (R1).

Process validation

The validation studies for GSK3336223 are satisfactory. The applicant has submitted adequate manufacturing process data. The methods used for analysis are sufficiently set out in different parts of the dossier.

Manufacturing Process development

An overview over the process development history is given in the dossier.

Improvements to the safety of certain raw materials were made.

The development studies were presented as part of the process development and the conclusions are accepted and support the comparability studies.

Comparability of the vector

The applicant has conducted a pairwise comparability assessment between each consecutive process development stage. Analysis focused on potency, identity, genetic stability and safety.

A concern was raised in relation to the formation of viral aggregates. The applicant has committed to develop an assay and monitor viral aggregates.

Vector control strategy

The applicant has presented a control strategy which is considered satisfactory and given a list of CQAs. Rationales for the testing of certain CQAs are provided. The designation of CPPs and IPCs is overall agreed with. The applicant has committed to ongoing process verification during the product life cycle.

Characterisation

The applicant has conducted the characterisation of the vector based on the proposed release criteria combined with additional tests, in particular an analysis of vector stability and integration site analysis.

An analysis was done to confirm vector integrity and to demonstrate that the provirus is stable.

For the analysis of product related impurities, the applicant has sufficiently justified that the absence of a test for viral aggregate formation is not a safety risk and has further committed to develop an assay to test for viral aggregates post licensure.

The identification of relevant process-related impurities is agreed.

Specification

The specifications for retroviral vector GSK3336223 are detailed in the dossier. The applicant has outlined the justification for the setting of specifications, based on clinical and manufacturing experience.

Analytical procedures

The applicant has given an overview over the development of the release assays over time and provided descriptions of the most current assays. Methods for analytical procedures are clear and give sufficient details of the assays in question. Validation summaries and reports of non-Ph.Eur. assays have been submitted and qualification summaries for a number of Ph.Eur. methods have been provided.

It was noted that all analytical tests that have a viral transduction component are carried out with one specific transduction agent instead of another transduction agent, which was identified as a concern. During assessment this concern could be alleviated by an analysis and it was not recommended to change the transfection reagent as this would require a revalidation of all relevant assays.

The applicant has submitted sufficient information on the reference standards that are used, and details of the manufacture and qualification of each of the reference materials are provided.

Container closure

The applicant has adequately described the container closure system for the vector. Acceptance specifications for the packaging components have been set out. Upon request, the applicant has provided additional information on the container closure and leachables, with respect to storage at -70°C.

Stability

The applicant has presented real time stability data for GSK3336223 supported by accelerated stability data. In conclusion tests carried out are appropriate and the requested shelf-life is acceptable.

Part 2: Transduced CD34+ cells with vector (GSK2696273)

Manufacture, characterisation and process controls

Manufacturing process

The active substance transduced CD34+ cells with vector (GSK2696273) is manufactured at MolMed S.p.A., Via Olgettina, 58, 20132 Milano, Italy. To generate the active substance, autologous CD34+ cells are isolated and transduced with the retroviral vector GSK3336223 (see part 1).

Harvest of patient bone marrow is performed at the San Raffaele Hospital, Milan, Italy in accordance with the EU Directives 2004/23/EC and 2006/17/EC. The bone marrow material is transferred from the syringe into another container and the number of CD34+ cells is assessed and the cells undergo microbiological testing.

Bone marrow aspirate is received into the facility, diluted and mononuclear cells (MNCs) are isolated for further processing. Cells are then washed, isolated and transduced with the GSK3336223 retroviral vector.

The applicant has provided an adequate description of the manufacturing process. This includes a sufficiently detailed description of the manufacturing process steps, flow-charts and tables of PPs, CPPs, IPCs and IPSs, which give operating ranges, set points and acceptance limits.

A sufficient description of the batch size and numbering system has been provided.

Control of Materials

The applicant has provided information on the control of biological raw materials, which includes supplier's qualification and audits. A list of the non-biological raw materials has also been provided indicating the use, origin and acceptance criteria.

A confirmation that no substances of human or animal origin are used in the manufacture has also been provided.

Donor Bone Marrow is collected at a JACIE accredited facility in accordance with JACIE standards. Suitable collection procedures and acceptance criteria have been defined. Compliance with EU Directive 2004/23/EC and EU Directive 2006/17/EC is demonstrated.

Process validation

The applicant has presented process performance qualification (PPQ) data from a number of consecutive batches of active substance.

Overall the PPQ has been carried out satisfactorily and data were provided for all PPs, CPPs IPCs and IPS as well as additional characterisation data. The results demonstrate an acceptable level of process consistency. All process parameters were met and acceptable process control was demonstrated. Total cell numbers are variable, which is expected due to the variability of the starting material.

Process development

The applicant has given an overview of the manufacturing process changes over time.

Process control strategy:

The applicant has provided a justification for each CQA. Process parameters were evaluated. Summary rationales are provided for each CQA, together with an overview of the analytical method and control strategy.

Concerns were initially raised on the control strategy for transduction efficiency. However, the applicant has introduced an assay, which specifically characterises the population of cells in the active substance.

Characterisation

Characterisation of the transduced CD34+ cells in terms of identity, purity and potency has been assembled through comparability studies and additional characterisation studies.

Characterisation methods together with their description and attributes are detailed.

Process-related and product-related impurities that could potentially impact safety and/or efficacy of Strimvelis have been identified and analysed.

Specification

The applicant proposes a release testing strategy in two stages. This approach is considered acceptable in the light of the specific properties (i.e. shelf life) and application of the product.

Potency is tested with a combination of a functional assay, an assay for vector copy number and an assay for transduction efficiency. The functional test measures increases in functional activity of transduced CD34+ cells as a result of the transduction process compared to a baseline detected in non-transduced cells.

During the procedure, additional information was requested for the reference standards used, including details on the manufacture and qualification for each of the reference materials. The applicant has committed to establish a new single reference standard with retroviral vector manufactured according to the commercial process. This has been accepted.

The description of analytical methods presented is acceptable. The applicant has given an overview of the development of analytical method where this was appropriate, as well as validation studies. The requested validation reports have been provided.

The batch analytical data have been presented, and are within specification.

Stability

The proposed shelf life for the active substance is 6 hours, when stored under a laminar hood with artificial light. The applicant has conducted a satisfactory stability testing programme and included relevant stability indicating parameters. The requested active substance shelf life is acceptable.

Comparability exercise for the Active Substance

The applicant used a side by side analysis and compared commercial process AS prepared with commercial process vector to clinical process active substance prepared with clinical process vector. Comparability of the proposed commercial production process with the Process, which was used to manufacture clinical trial material, has been demonstrated.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The finished medicinal product is Strimvelis 1-10 million cells per mL dispersion for infusion. The active substance contained in Strimvelis is autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector GSK3336223 that encodes for the human ADA cDNA sequence. The product is also referred to as GSK2696273 Dispersion for Infusion by the applicant.

The finished product is supplied in one or more EVA bag, 50 mL nominal fill, with a Luer spike interconnector closed with a Luer lock cap. T

The composition of the finished product is presented in the following Table.

Table. Composition of the finished product

Component	Quantity	Function	Reference to Standard[1]
Autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence[2]	1-10 million CD34+ cells/mL	Active substance	In-house
0.9% w/v Sodium Chloride Infusion	0.9% w/v	Tonicity adjustment and vehicle	Medicinal product (EU)

Note:
1. Further details are provided in S.4.1. Specification and P.4. Control of Excipients for the active substance and excipient, respectively.
2. The active substance is also referred to as GSK2696273

The only excipient used in the formulation of GSK2696273 Dispersion for Infusion is 0.9% w/v Sodium Chloride Infusion (0.9% sodium chloride), which is routinely used for intravenous infusion. The purpose of this excipient is to provide a cell suspension for infusion.

The final volume of each batch of finished product depends on the quantity of haematopoietic/progenitor cells obtained from the active substance manufacture and is patient-specific.

Changes during the manufacturing process development are considered moderate.

Container closure system

GSK2696273 Dispersion for Infusion is filled into sterile, single use, ethylene vinyl acetate (EVA) bags at 50 mL nominal fill.

Once filled with GSK2696273 Dispersion for Infusion, the EVA bag(s) are placed into an outer box for transportation. The secondary packaging is used to protect the bags and includes a bag holder device to prevent damage during transportation.

The applicant has given a satisfactory description of the primary and secondary container closure system.

The compatibility of the finished product with the container closure and administration set has been sufficiently demonstrated.

Manufacture of the product and process controls

Manufacture of the finished product takes place at MolMed S.p.A., Via Olgettina, 58, 20132 Milano, Italy.

The active substance is concentrated and the cell pellet is washed and resuspended in 0.9% w/v Sodium Chloride. The cells are then diluted to obtain a concentration of 10×10^6 total viable cells/mL. The finished product cell suspension is transferred from the centrifuge tube into the EVA bag.

The manufacturing process for the finished product has been sufficiently described, flow charts are provided and CPPs and their acceptable ranges are given.

The information set out under control of critical steps and intermediates is overall adequate.

Batch analytical data have been presented for all batches manufactured and the data are in conformity with viability and cell concentration.

The applicant has presented data on the impurities in the active substance section and no other impurities are introduced as part of the finished product manufacturing process.

Stability of the product

Based on available stability data, the shelf life of 6 hours stored at 15-30°C as stated in the SmPC is acceptable.

Real time/real condition stability data for finished product stored for twelve hours are presented for three batches of GSK2696273 Dispersion for Infusion manufactured at MolMed S.p.A using the commercial manufacturing process (A4) with the EVA bag as the primary container.

Overall the stability data submitted support the requested shelf life.

Comparability exercise for finished medicinal product

The main data set for the comparability of the finished product is presented in the active substance section and is based on product manufactured from the active substance comparability batches.

Adventitious agents

The applicant has provided sufficient information to demonstrate that the TSE risk of the product is managed via appropriate sourcing of FBS. A risk assessment of the parental cell line was conducted.

Detailed adventitious agents testing were conducted, and the results have been provided. The information is acceptable and in compliance with ICH Q5A (R1) guidance.

The applicant has provided a risk assessment for the detection of bovine and porcine circovirus, which provide reassurance of the viral safety of the material.

GMO

The active substance consists of autologous haematopoietic stem cells which contain cells genetically modified to express the enzyme ADA. Transduction of the cells is achieved with the use of a MoMLV based genetically modified retrovirus. Safety features of the virus are described above and an environmental risk assessment has been presented by the applicant with respect to the risk of release of residual infectious viral particles in the finished product. This assessment is discussed in more detail in the non-clinical part.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The quality part of the dossier is acceptable. Initially several other concerns have been identified and the applicant has provided additional information as requested and amended the dossier accordingly.

In particular the applicant has provided important new data on the transduction efficiency assay, which demonstrate that transduction efficiency observed during the clinical study is within the same range as that obtained for product from the proposed commercial manufacturing process.

The applicant has introduced a number of changes both to the vector production and to the manufacture of the active substance and finished product in preparation for the commercial manufacture. These changes are welcomed as they have increased the quality of the product by reducing animal derived substance or the use of substances with reduced adventitious agent risk, improving sterility assurance and reducing process residuals in the finished product.

In support of the commercial process, the applicant has also submitted a comparability study. In light of the additional information submitted by the applicant, it is considered that the commercial process is comparable to the clinical process.

Clinical batches cover a fairly wide range of specifications. However as Strimvelis has been in clinical development for a long time with a demonstrated favourable efficacy and safety record the acceptance of the release specifications on the basis of clinical qualification is considered acceptable. In this respect the applicant also demonstrated that release of future commercial batches is within the clinically qualified boundaries, despite changes introduced for the proposed commercial manufacturing process as described above.

The applicant has agreed to a number of post-approval recommendations aimed at completing the set of specifications, characterisation tests for the retroviral vector, stability data and to introduce the validated transduction efficiency test.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall Quality of Strimvelis is considered acceptable. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The manufacturing process of the active substance is adequately described, controlled and validated. The active substance is well characterised and appropriate specifications are set. The manufacturing process of the finished product has been satisfactorily described and validated. The quality of the finished product is controlled by adequate test methods and specifications. Adventitious agents' safety including TSE safety has been sufficiently assured.

No measures necessary to address the any quality developments issues have been identified that have

a potential impact on the safe and effective use of the medicinal product.

The CHMP endorse the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT/CHMP recommends the following points for investigation: 10 recommendations aimed at completing the set of specifications, characterisation tests for the retroviral vector, stability data and to introduce the validated transduction efficiency test, are agreed between the Applicant and the CHMP/CAT. The details of these recommendations are deleted as they are considered commercially confidential.

The CHMP endorse the CAT assessment regarding the recommendation(s) for future quality development as described above.

2.3. Non-clinical aspects

2.3.1. Introduction

Strimvelis is a gene therapy medicinal product developed the treatment ADA-SCID when no suitable HLA-matched related stem cell donor is available. Strimvelis consists of autologous CD34+ cell fraction containing CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence. Transduced CD34+ cells are expected to engraft into bone marrow, divide, and differentiate allowing reconstitution of immune and metabolic functions, improving immune protection, systemic detoxification and survival.

The non-clinical study reports, summaries and bibliographic data are included for pharmacology, pharmacokinetics, and toxicology. Due to the innate nature of GSK2696273 and limitations associated with investigating the clinical vector in standard animal models, most nonclinical studies were deemed unlikely to provide meaningful insight into the clinical safety of GSK2696273 or were considered not to be feasible. Therefore, certain nonclinical studies, such as safety pharmacology, select pharmacokinetic studies, e.g. absorption, distribution, and excretion; genotoxicity, reproductive toxicity, and other studies are not considered relevant. As Strimvelis contains GMOs within the meaning of Directive 2001/18/EC, a GMO Environmental Risk Assessment (ERA) has been provided.

The nonclinical studies conducted as part of the development of GSK2696273 employed the use of the GSK3336223 vector; however, it was not practical or ethical to harvest patient bone marrow (BM) to obtain sufficient CD34+ cells for use in all nonclinical studies. Therefore, some nonclinical test materials were developed with cells from alternate sources. Hence, whilst some pharmacology studies utilised patient bone marrow cells, other studies used peripheral blood lymphocytes (PBLs) or CD34+ cells derived from healthy human umbilical cord blood (UCB) or murine lineage negative (lin-) BM cells (considered the murine equivalent of human CD34+ cells) transduced with the GSK3336223 vector (or vectors with similar backbones). Subsequently, the following company codes apply: GSK3235757A stands for Healthy human UCB transduced with GSK3336223 vector; GSK2959346 stands for Murine lin- BM cells transduced with GSK3336223 vector.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The early nonclinical development of GSK2696273 was conducted by TIGET and the summary of the nonclinical studies (see below) were made available to the applicant as the only source of data. The data is not contained in formal standalone reports. However, it is stated that that information is superseded by the more robust descriptions in the literature. Accordingly, two publications [Ficara, 2004] and [Cassani, 2008] are considered the primary source of the pharmacology data. These early development results are based on a small numbers of samples using early batches of autologous $CD34^+$ enriched cell fraction that contains $CD34^+$ cells transduced with retroviral vector encoding for human ADA cDNA (GSK2696273) in non-validated assays. For the purposes of this application, the drug product utilised in this early development stage is considered representative of the product now proposed for marketing.

The summary report provides discussion on the following points:

- *In vitro transduction efficiency*: CD34+ cells obtained from umbilical cord blood (UCB) from healthy human donors or BM from ADA-SCID patients were transduced with the GSK3336223 vector. The full range of TEs on CD34+ cells across all nonclinical pharmacology studies was 14% to 45%. These data all served to demonstrate that GSK3336223 vector is able to consistently transduce cells from various sources.
- *In vitro vector copy number (VCN):* Polymerase chain reaction (PCR) confirmed vector DNA in CD34+ cells from healthy donors or ADA-SCID patients transduced with GSK3336223 viral lots. VCN in the transduced CD34+ cells ranged from 0.14 to 2.2 (measured by quantitative PCR) and did not correlate with vector titre or transduction efficiency. Across all these experiments VCN ranged from 0.3 to 3.2 and demonstrated a good correlation with viral titre but not transduction efficiency.

  In a further study, VCN ranged between 0.12-1.87 in 5 clinical $CD34^+$ cell batches. In this study transduction was carried out in tissue culture plates; for the first transduction the vector was preloaded onto retronectin-coated plates. For the second and third transduction cycle the vector was diluted in culture medium at the concentration of $0.5x10^6$ cells/ml.
- *In vitro functional activity*: GSK3336223 supernatant (3 lots) was tested for the ability to transfer ADA into murine lineage negative (ADA-/-) progenitor cells from ADA-deficient mice. There was no ADA activity in untransduced cells; however, one week after transduction, ADA activity was detected (15% to 36% of normal BM ADA). Additionally, CD34+ cells from the BM of ADA-SCID patients were transduced with the GSK3336223 vector. ADA expression, measured by intracellular fluorescence activated cell sorting (FACS) analyses, was detectable (20% to 39%) in transduced CD34+ cells after short-term in vitro culture and absent (as expected) in un-transduced cells.

  Unpublished data (Cassani et al., in preparation) cited by the applicant state that T-cell from ADA-SCID patients have impaired proliferative responses and Th1/Th2 cytokine production, in association with a defect in TCR signalling, transcription of cytokine genes and kinase activation (CREB, MAPK). Consequently, details of a further investigation are presented: human ADA-deficient T cell lines transduced with the GSK3336223 vector demonstrated a similar proliferative capacity and production of cytokines when compared with healthy donor cells, presumably demonstrating 'functional' restoration following gene transfer, further supporting the rationale for gene therapy in ADA-SCID patients.

- *In vivo engraftment:* Because ethical restrictions of harvesting of human bone marrow for experimental purposes limit *in vivo* studies of the human hematolymphoid system, substitute human to small animal xenotransplantation models has been employed. Although existing models sustain only limited development and maintenance of human lymphoid cells and rarely produce immune responses it has been reported that intrahepatic injection of CD34 human cord blood cells into conditioned newborn recombinase activating gene 2 (RAG2)-gamma chain immunodeficient mice (a mutant strain that lacks B, T, and NK cells) permits engraftment of human CD34+ stem progenitor cells and differentiation in different hematopoietic lineages to assess the biodistribution of genetically modified human CD34+ cells [Traggiai, 2004]. Therefore, this model was employed for GSK3336223-transduced CD34+ cells from the BM of ADA-SCID patients. The summary states that results demonstrated that these transduced cells engrafted into BM, differentiated normally into T (CD4+ and CD8+) and B (CD19+) lymphocytes in lymphoid organs (thymus, BM and spleen) and circulated in peripheral blood.

The literature data on primary pharmacology supersede the above summary reports. Two publications (*IL-3 or IL-7 increases ex vivo gene transfer efficiency in ADA-SCID BM CD34+ cells while maintaining in vivo lymphoid potential*; Ficara et al, 2004) and (*Altered intracellular and extracellular signalling leads to impaired T-cell functions in ADA-SCID patients;* Cassani et al, 2008) are considered by the applicant the primary source of pharmacology data.

- *Ficara et al, 2004*

In vitro: CD34+ cells obtained from UCB or BM (healthy donors and ADA-SCID patients) were used to optimise gene transfer and transduction conditions to maintain stem/progenitor cell properties. Cells were grown in serum free media and were subjected to three rounds of transduction with RV, in the presence of fibronectin fragment (retronectin) to favour co-localisation of target cells with vector particles. To assess their multilineage differentiation potential, unmanipulated, untransduced and transduced CD34+ cells were used in clonal assays of differentiation. Several combinations of cytokines and culture medium were tested using BML-1 vector (RVs with the same backbone as GSK3336223) encoding a cell surface marker gene (ΔNGFR) to assist with detection, quantification, and purification of transduced cells. Optimal conditions for *ex vivo* gene transfer and maintenance of haematopoietic progenitor cells included cytokine stem cell factors [thrombopoietin, FMS-like tyrosinekinase 3 ligand as well as interleukin (IL)-3] and supplementation of medium with 4% foetal calf serum. Cultured CD34+ cells maintained the ability to differentiate into myeloid and erythroid cells (in clonogenic assays) and to differentiate into B and natural killer (NK) cells (in lymphoid differentiation assay). Up to 50% of ex vivo cultured CD34+ cells maintained the CD34+ cell phenotype.

In vivo: Immunodeficient mice were implanted with human hematolymphoid tissues (SCID-hu mice), irradiated and transplanted with a single dose of cultured/transduced CD34+ cells. The transduced CD34+ cells engrafted in mice, produced vector-marked and transgene expressing T and B cells and maintained in vivo lymphoid reconstitution capacity. The *ex vivo* gene transfer and culture conditions did not compromise the reconstitution potential of stem/progenitor cells in human-mouse chimeras.

Ex vivo: Efficient correction and engraftment of hematopoietic stem/progenitor cells with lymphoid potential is the main goal of gene therapy for primary immunodeficiencies, including ADA-SCID. The first ADA/SCID clinical protocol for Study Number AD1117054 was based on the optimised transduction conditions from the nonclinical studies described above. BM was harvested from the patient, 4 and 11 months after receiving GSK2696273. Purified CD34+ cells were then injected into SCID-hu mice. Eight weeks post dose, engraftment levels of donor cells ranged from 85% to

98%. CD34+ cells gave rise to T and B lymphoid progeny with all the thymic subpopulations present in the expected ratios. Vector-containing cells were measured in B (up to 15%) and T (up to 31%) lymphocytes as well as T cell lines of donor origin (up to 42%). There was no increase in the proportion of transduced cells compared to the initial frequency of the CD34+ cell population, most likely due to the lack of a selective advantage in the normal, detoxified microenvironment of the SCID-hu mouse. However, the frequency of transduced T cells increased 4 weeks after culture, suggesting an in vitro survival advantage.

- *Cassani et al, 2008*

Biochemical/functional studies: BM samples collected from three ADA/SCID patients under clinical studies AD1117054, AD117056 and AD115611 were harvested before and 10 to 30 months after dosing GSK2696273. Cells from ADA-SCID patients generally have decreased activity in the S-adenosylhomocysteine hydrolase (SAHH) enzyme, high susceptibility to apoptosis after exposure to adenosine and impaired proliferative responses and Th1/Th2 cytokine production (T cells). T cell lines (post dose) expressed the ADA protein (intracellular FACS analysis). ADA activity was detected in all patients, albeit not to normal levels in all patients. SAHH was restored in T cells, suggesting that transgene expression reached therapeutic levels sufficient to correct the metabolic defect. Intracellular signalling was also corrected in T cell lines, resulting in restoration of normal proliferative responses and production of cytokines. Apoptosis was normalised in gene-corrected T cells. However, the authors concluded *'that it remains to be established whether ADA expressed by circulating or resident gene-corrected hematopoietic cells will be sufficient to correct long-term the nonimmune manifestations of the disease'.*

Secondary pharmacodynamic studies

No secondary pharmacology studies were considered feasible in animals or relevant to the administration of GSK2696273 to humans. Due to the specific, unique and ubiquitous role of the ADA enzyme, this is unlikely to cause any off-target activity.

Safety pharmacology programme

As per the applicant's statement, no safety pharmacology studies were considered feasible in animals or relevant to the administration of GSK2696273 to humans. This was due to the fact that GSK2696273 is an autologous, patient derived product expressing a single therapeutic gene (ADA) as well as a NeoR selection marker gene. The therapeutic gene product is an intracellular enzyme with ubiquitous expression in healthy ADA+/+ humans. ADA protein expression and enzymatic activity following transduction with GSK2696273 are below or equal to physiological levels. Therefore, the applicant concludes that even if inadvertently expressed in non-target cells (e.g. heart) there are unlikely to be any adverse functional consequences. Although it is assumed that within the target cell population there will be cells with a higher than average VCN that might lead to overexpression of the ADA gene, the applicant clarified that clinical enzymatic activity levels in patients reported thus far does not suggest enzymatic activity above that reported in healthy volunteers where ADA expression is ubiquitous unlike in the gene therapy setting (transduced cells only). The applicant also addressed the CAT/CHMP's question on the likelihood of secondary transduction (non-target cells) due to the presence of residual vector in the plasma. Steps taken during the manufacturing process (e.g. washing residual vector from DP and subsequent reported levels of residual vector being below the level of quantification) are expected to allow for the impact on safety and efficacy to be considered low. Further reassurance is given by the fact that no transduction of non-target cells was shown in the non-clinical biodistribution study.

Considering the arguments regarding the likelihood of achieving even physiological levels overall, the CAT/CHMP reassured that adequate precautions and investigations have been employed (e.g. manufacturing process/biodistribution study).

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were performed with GSK2696273 and this was considered acceptable to the CAT/CHMP.

2.3.3. Pharmacokinetics

Since Strimvelis is a gene therapy medicinal product, the standard programme of pharmacokinetic studies for a new chemical entity is not strictly applicable. Furthermore, due to the nature of Strimvelis, most studies were considered by the applicant as neither feasible nor informative for administration to humans; therefore, the majority of standard nonclinical pharmacokinetic studies were not conducted (e.g. absorption, excretion, metabolism, drug interaction). Nevertheless, the toxicity of the concomitant use of busulfan was discussed.

Subsequently, the results of two bio-distribution studies were submitted using the NSG mouse based on the reports presented that indicate this is a valid model for immunodeficient mice, which can be used as recipient of HSPC transplantation, with longer survival rates and reduced frequency of spontaneous tumours as compared to ADA-/- mice. Transplantation of haematopoietic stem/progenitor cells (HSPC) into NSG mice requires conditioning via chemotherapy or irradiation, in order to provide depletion of endogenous bone marrow and to allow the engraftment of donor HSPC. Studying biodistribution in immunodeficient mice given human cord-blood derived-product generally suffices to address the expectations for kinetic data.

In the pilot study, fluorescence activated cell sorting (FACS) and vector copy number (VCN) analyses in group 1 (received busulfan treatment as pre-conditioning) and group 3 (irradiation pre-conditioning) showed successful engraftment of transduced UCB cells in NSG mice both after busulfan conditioning and irradiation. The percentage of human lymphocytes (mainly B cells) was relatively stable in the peripheral blood at 8 and 10 weeks after transplantation. This finding is consistent with reports in literature showing efficient reconstitution of human immune cells with preferential and earlier development of B cells in NSG mice. The bone marrow showed human haematopoietic stem cell engraftment with multi-lineage differentiation of lymphoid and myeloid cells. In the peripheral lymphoid organs, human T- and B-cell differentiation could be observed in the thymus and spleen respectively. Group 1 showed significantly higher engraftment levels as compared to group 3 which is a finding supported by bibliographic data.

The VCN measured in whole blood and BM was variable but overall comparable between both groups and time points. A higher VCN was observed in mice in group 1 (conditioned with busulfan) in comparison with group 3 (irradiated). With the exception of thymus samples from one mouse, which failed engraftment, VCN was detected in 93% of samples. No significant statistical differences in VCN were observed in PB or lymphoid organs.

Subsequent to these results, busulfan was selected for pre-conditioning of mice in the definitive study. In this study, three groups were utilised (RV transduced human UCB cells, mock-transduced cells and busulfan vehicle only controls). The last observation point was 16 weeks and no clinical signs attributable to treatment were reported. Busulfan-related deaths occurred in in the first month of treatment but not in the control animals. Successful engraftment and immune reconstitution were recorded in both groups of NSG mice transplanted with RV(GSK3336223)-transduced or mock-transduced cells. Histopathological analyses on target lymphoid organs assessed the biodistribution of

human cells in busulfan-conditioned and transplanted NSG mice. Reconstitution of the haematopoietic system in successfully transplanted mice was observed secondary to transplantation and engraftment (increased white pulp cellularity in the spleen and bone marrow). As expected from the hematopoietic nature of the test item and its distribution in the blood stream, the presence of low levels of human cells was also detected by qPCR in non-target organs such as the brain, heart, kidney, liver, lung, muscle and gonads. The presence of RV specific sequences was assessed in whole blood, bone marrow, spleen and non-target organs of NSG transplanted mice. In blood, VCN was generally lower (in comparison to the VCN measured in the test Item batch before infusion) after administration, but comparable between time points and between sexes.

Low amounts of human DNA and RV sequences were detected in the gonads. The applicant explained that this was likely due to the contamination by residual blood containing transduced human cells or the presence of resident monocyte-derived cells of human origin. DNA sequences specific to the human ADA gene were detected in 4/18 gonad samples harvested in group 3 at level of the definitive biodistribution study above the LOQ indicating exposure in the gonads. Considering the Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors, the applicant has provided a discussion related to risk of germline transmission (see section on Reproductive Toxicology).

No nonclinical studies were performed to assess vector shedding after GSK2696273 administration. As reflected in the valid guidance (EMEA/CHMP/GTWP/12459/2006, Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products), biodistribution, persistence, mobilisation and shedding should be assessed. The applicant states that in humans, the possibility of vector shedding (e.g. via urine or other body fluids) after Strimvelis administration is extremely low as the number of infectious vector particles was below the LLQ. As this RV is derived from murine cells lines, added reassurance is given due to reports that such RVs are known to be inactivated by human serum via the classical complement cascade. Additionally, as Strimvelis will be administered only once, this limits further any possible vector shedding. It is also stated that no evidence of replication competent retrovirus (RCR) was detected in clinical trials with GSK2696273.

In conclusion, investigations of distribution of transduced cells in a relevant non-clinical model showed comparable capacity of transduced and mock-transduced CD34+ cells to engraft and reconstitution in vivo was demonstrated confirming that transduced cells are not directed to specific sites. Successfully transplanted mice showed reconstitution of the haematopoietic system secondary to transplantation and engraftment, i.e. increased cellularity in the white pulp of the spleen and BM. Low levels of human cells were detected in non-target organs and this is not unexpected due to the hematopoietic nature of the test item. Low levels of human DNA and RV sequences were detected in the gonads and other non-target organs, likely due to contamination by residual blood containing transduced human cells and the risk that may be associated with the potential for germline transmission are addressed in the toxicology section below. Of note, histological changes in the testes and ovaries (i.e. atrophy), considered to be related to the administration of busulfan, were observed in both groups that received conditioning. The observation time to determine survival, engraftment and clinical signs in this combined biodistribution/toxicology study was 16 weeks. Biodistribution analysis did not show any evidence of transduction of non-target cells: vector DNA was only detected in the presence of donor cells.

2.3.4. Toxicology

Single dose toxicity

Due to the nature of GSK2696273, single dose toxicity studies were not conducted. It is stated that GSK2696273 consists of patient-derived cells expressing a housekeeping gene at or below physiological levels. Therefore selected general toxicity endpoints were assessed in the definitive biodistribution study in mice, see table below.

Type of Study /Id	Species (strain)	No./Sex /Group	Route	Test Item b	Single Dose (Total no. cells or no. cells/kg)	GLP
Biodistribution (general toxicity endpoints) 2014N198496 (TM021 and M30711G)	Mouse (NSG)	10M/10F 5M/5Fa	IV	Mock-transduced or GSK3235757A	2 $x10^5$ cells (10 $x10^6$ CD34+ cells/kg)	Yes

Key: a = Control mice received busulfan vehicle only. b = Mice also received IP busulfan at 40 mg/kg (total dose divided over 2 days). c = In-life portions of the study were conducted at HSR TIGET, Ospedale San Raffaele to GLP. Sample analysis (qPCR) was conducted at Covance to GLP. Histology and microscopic pathological examination was conducted by GlaxoSmithKline to GLP. Preparation of the test items (transduced and mock-transduced) was performed by MolMed and was not conducted to GLP. GSK3235757A = Umbilical cord blood haematopoietic stem cells (CD34+) transduced by GSK3336223 RV. GSK3336223 = RV that encodes for the human ADA cDNA sequence. GLP = Good laboratory practices. ADA = Adenosine deaminase. IP = Intraperitoneal. IV = Intravenous. NSG = NOD-SCID immunodeficient mice. RV = Retroviral vector.

The test item used in the biodistribution studies (referred to as GSK3235757A) comprised of CD34+ cells derived from healthy human UCB transduced with GSK3336223 vector and is accepted as representative of Strimvelis. In this study, NSG mice (n=10/sex/group) were pre-conditioned with busulfan [40 mg/kg total dose over 2 days; intraperitoneal (IP)] prior to dosing transduced cells (single intravenous (IV) dose (2 $x10^5$ cells) of CD34+ cells derived from human UCB transduced with either the GSK3336223 vector (GSK3235757A) or mock-transduced. Control mice (n=5/sex) were given busulfan vehicle only. Clinical observations, body weight, macroscopic examination; microscopic examination of bone marrow (long bone), spleen, thymus, brain, heart, kidney, liver, lung, muscle, gonads and macroscopic abnormalities were recorded.

One male and 3 female mice (Days 8 and 15, 25, 28, respectively) in the mock-transduced group and 2 males (Days 17, 24) in the GSK3235757A group were found dead or euthanised. These deaths were attributed to acute toxicity of the pre-conditioning treatment (busulfan) or the transplantation procedure.

One mouse in the GSK3235757A group developed lymphomas; however, this was not considered treatment related as there was no confirmed engraftment of human cells (<1% of BM CD45+ cells) and no vector DNA sequences were detected in this mouse. The changes observed in the haematopoietic system were considered related to the transplantation and engraftment of UCB CD34+ cells and their progeny in NSG mice that have a severe depletion of lymphoid cells. There was an increased incidence and severity of vacuolation of alveolar macrophages in mice receiving mock-transduced cells or GSK3235757A when compared to controls. Since this was observed in both groups it was not considered related to treatment with GSK3235757A.

Very marked atrophy of the testicular seminiferous tubules and marked or very marked atrophy ovaries with no oocytes present were observed in virtually all successfully engrafted animals (>1% human CD45+ BM cells) from both transplanted groups. This was independent from GSK3235757A or control, but is consistent with an effect of busulfan conditioning. Since exposure to busulfan was not measured in mice in this study a comparison with human exposure cannot be made. However, whilst the busulfan dose relative to body weight used in this study was higher than the dose used for pre-conditioning in patients prior to treatment with Strimvelis, the doses used in mice and in patients on

mg/m^2 basis are equivalent. In addition, busulfan is known to have the potential for gonadotoxic effects in humans. The exact severity of any gonadotoxic effects in patients cannot be predicted with certainty.

The use of NSG mouse strain (deficient in mature T/B/NK cells) for the non-clinical biodistribution/single-dose toxicology study after engraftment of CD34+ cells derived from healthy human UCB, transduced with GSK3336223 vector, was endorsed by the CAT/CHMP prior to MAA submission. This is due to the fact that survival rates of this mouse strain extend to >16 months, allowing for long term toxicology observations, that would otherwise not be possible in other immune-compromised mouse strains (with NOD backgrounds). Strimvelis will be administered to ADA-SCID patients as a single IV infusion with a dose range of 2 to 20 x10^6 CD34+ cells/kg. In this study, mice were administered 10 x10^6 CD34+ cells/kg (approximately the midpoint of the dose range to be infused in human subjects) without test-item-related adverse effects. The CAT/CHMP noted that the changes observed in the haematopoietic system were not considered related to the treatment.

As no safety margins calculation was made by the applicant, the CAT/CHMP requested a clarification. The doses administered provide only safety margin of approximately half the highest dose administered in humans (20 x106 CD34+ cells/kg). The product administration did not result in apparent dose-limiting toxicities observed in the clinical programme and there was a low risk of overexpression. Although it is not possible to establish a safe VCN, this has been maintained at a lowest possible degree that results in sufficient transduction efficiency. Taking into account the proposed indication and the evaluation of the risk of use of the product, the CAT/CHMP considered this issue resolved.

Repeat dose toxicity

Due to the nature of Strimvelis, repeat dose toxicity studies were not conducted since GSK2696273 consists of patient-derived cells that are intended to consist of the ADA gene, to allow for expression of the related ubiquitous protein at or below physiological levels. Furthermore, the intended clinical use is once only, with longer term exposure of the resultant protein expected. This is accepted by the CAT/CHMP.

Genotoxicity

Conventional genotoxicity assays are inappropriate to detect insertional events and would not provide additional information to inform a quantitative risk assessment in humans and hence no studies have been conducted.

Carcinogenicity

The use of γRV mediated gene therapy has been associated with insertional mutagenesis/oncogenesis with in several different gene therapy trials. For ADA-SCID, analysis of γRV integrants has been analysed in a similar way to the studies for the other immuno-deficiencies and shows a very similar insertional profile. Integrations are found predominantly in and around the transcriptional start site of genes and insertions have been found in genes associated with cell cycle control, cell signalling and near known oncogenes such as LMO-2. Due to these known risks, the applicant was advised in 2007 to produce data from animal studies and/or discuss the applicability of all available animal models for insertional oncogenesis with the view of monitoring post-treatment for 12 months (the latency period for hematopoietic malignancies can be >6 months). The applicant discussed why it was not possible to generate suitable experimental conditions to allow for long term analysis of the in vivo carcinogenicity potential of the test item.

Following administration of GSK2959346, the achieved VCN was low and there was no long term engraftment of transduced cells in mice. To increase the likelihood of long term engraftment, alterations were made to optimise transduction conditions for murine cells and for engraftment. The impact of different transduction conditions of cells was tested in vitro and in vivo; the effect of the genetic background of mouse cells and the type of vector used were also examined. In the short term, transduction of murine cells and engraftment into mice was achieved. However, the applicant stated that it was not possible to demonstrate stable, long term engraftment of transduced cells under any condition tested in these pilot studies (with the exception of cells transduced with a lentiviral vector carrying ADA cDNA). The attempted in vitro and in vivo approaches to allow for the assessment of risk of clonal expansion and tumour arising from genetically modified cells, although unsuccessful, are accepted as comprehensive investigations. The study description indicates they were scientifically thought through and no further investigations are warranted at this time.

None of 18 subjects treated with GSK2696273 have developed leukaemia or MDS despite a median of 7 years and a maximum of 13 years of follow-up. Investigations for other haematological abnormalities that could be indicative of clonal abnormalities including B cell immunoglobulin production, T-cell receptor V-beta repertoire, bone marrow morphology and immunophenotype, peripheral blood smears, and cytogenetic karyotype analysis showed no clinically problematic changes. The encouraging safety record for gamma retroviral vectors in ADA-SCID is supported by the finding that none of the 40 patients with ADA-SCID that have received gene therapy with either GSK2696273 (n=18) or other comparable gamma retroviral vectors (n=22) with an extended follow-up period have developed leukaemia.

In summary, in previous scientific advice, it was agreed that the tumourgenicity risk assessment for GSK2696273 could be based on clinical data and literature on similar vectors if tumourgenicity/general toxicity studies were unsuccessful in allowing for the determination of optimal conditions for a full tumourgenicity study. Conducted pilot studies indicate that a tumourgenicity study is not feasible since it was not possible to demonstrate long-term engraftment of transduced cells in mice. Although clinical data appear reassuring, thus far; from a non-clinical perspective the carcinogenic potential due to insertional mutagenesis and the potential for subsequent clonal expansion cannot be determined at the time of assessment. Accordingly, the SmPC states that carcinogenicity studies have not been conducted as no adequate animal model was available to evaluate the tumourigenic potential of Strimvelis due to the inability to achieve long-term engraftment of transduced cells in mice. In addition, the RMP was updated and the risk of malignancy will be assessed via adverse event reports received during the ongoing long-term follow up of study AD1115611 and the proposed patient registry.

Reproduction Toxicity

No reproductive or developmental toxicity studies were conducted with GSK2696273. Patients will receive a low dose of busulfan as pre-conditioning treatment. Busulfan has a known gonadotoxic effects in humans and animals and this was confirmed in the biodistribution study in mice. Some relevant toxicity endpoints, including histopathology of testes and ovary, were evaluated in the pilot and the definitive biodistribution study and the presence of the transgene was reported. No juvenile studies were deemed necessary.

Toxicokinetic data

Due to the nature of the product, no specific toxicokinetic studies have been conducted.

Local Tolerance

Local tolerance studies were not considered necessary by the applicant and the CAT/CHMP, as stem cell treatment administered IV is a well-established clinical therapy and transduction of autologous cell does not alter the physicochemical properties of the cells.

Other toxicity studies

Not applicable

2.3.5. Ecotoxicity/environmental risk assessment

Directive 2001/83/EC, as amended, and Regulation 726/2004 require that the applicant evaluates the potential risk of the medicinal product to the environment. Unlike the ERA for medicinal products that are chemically derived, there is no action limit for GTMP about which threshold limit may be an environmental risk. There is no threshold for environmental effects; therefore a calculation of environmental risk is based on the probability of transmission of the GTMP from the patient to third party persons, animals, plants or the environment at large.

The ERA submitted is comprehensive and includes evaluation of both, the characteristics of GSK2696273 (Strimvelis) cells and of GSK3336223 (RV) viral vector particles, and of the potential exposure of people or the environment to Strimvelis dispersion for infusion. It is demonstrated that following transduction with GSK2696273 Dispersion for Infusion, the patient's cells are washed and previous drug product samples showed that little to no residual vector remained. However, in order to evaluate the worst-case scenario, it was assumed that minor traces remain in the product. All relevant GSK3336223-related manufacturing materials, though, are tested for recombinant virus formation in line with good manufacturing practice. Adequate safe-handling practises are in place limiting the possibility of healthcare professionals being exposed to Strimvelis. Moreover, the implementation of 'contained use' conditions minimises the possibility of the product contaminating the administration site or reaching the environment beyond the administration site. All associated waste is treated as a biohazard. Manufacture and administration are proposed at the same location and the number of patients is predicted to be ~20/year. The applicant adequately addressed the probability of introducing surface-bound retroviral particles. Further reassurance is given by the fact that Strimvelis is rapidly inactivated outside of appropriate host as the cells are autologous to the specific patient.

Finally, the ERA discussed the possibility of the formation of replication-competent retrovirus (RCR). Specifically, it is stated that this was not detected in clinical trials. In theory, the likelihood is deemed to be minimal, primarily due to the fact that the drug product has minimal homology with human endogenous retroviral sequences (HERV) and does not code for gag, pol or env sequences (required for RCR formation). The environmental risk posed by these theoretical figures is negligible. Based on the information provided in the ERA, Strimvelis poses a negligible risk to people and the environment.

2.3.6. Discussion on non-clinical aspects

Pharmacology studies indicated that the gene is expressed at the relevant site(s) at an appropriate level and that functional activity is realised. The absence of secondary and safety pharmacology studies is accepted on the basis that there studies were not were considered feasible in animals or relevant to the administration of GSK2696273 to humans. Pharmacokinetics addressed primarily the tissue distribution and duration of exposure. Biodistribution analysis did not show any evidence of transduction of non-target cells: vector DNA was only detected in the presence of donor cells. Low levels of human DNA and RV sequences were detected in the gonads and other non-target organs,

likely due to contamination by residual blood containing transduced human cells. The risk that may be associated with the potential for germline transmission is discussed in the toxicology section below. Histological changes in the testes and ovaries, considered to be related to the administration of busulfan, were observed in both groups that received conditioning. Toxicology was evaluated in a single dose (IV) mouse (NSG) biodistribution/toxicology studies. The pivotal study had a 4 month follow-up without test-item-related adverse effects and also evaluated the biodistribution of transduced CD34+ cells and their progeny to haematopoietic and non-haematopoietic organs, the possibility of transfer of vector to gonads and the histopathology of major organs. The test item used in the definitive biodistribution study was prepared from healthy human UCB CD34+ cells transduced with the GSK3336223 vector, manufactured using the commercial process. Histologically, the changes observed in the haematopoietic system were considered related to the transplantation and engraftment of UCB CD34+ cells and their progeny in NSG mice that have a severe depletion of lymphoid cells. This includes the increased white pulp cellularity in the spleen and the foci of immature looking cells observed in the bone marrow of the long bone. There was no evidence of thymic reconstitution in either mock transduced or transduced groups. The splenic and thymic observations have been previously reported in the literature.

Due to the nature of Strimvelis, repeat dose toxicity studies were not conducted since GSK2696273 consists of patient-derived cells that are intended to consist of the ADA gene, to allow for expression of the related ubiquitous protein, which the applicant states will be at or below physiological levels. Moreover, the intended clinical use is once only, with longer term exposure of the resultant protein expected. The absence of repeat dose toxicity is accepted by the CAT/CHMP.

The use of gamma-retroviral vector mediated gene therapy has been associated with insertional mutagenesis in 3 different gene therapy trials. Due to these known risks, the applicant was advised in 2007 to produce data from animal studies and/or discuss the applicability of all available animal models for insertional oncogenesis with the view of monitoring post-treatment for 12 months. In addition, long-term clinical monitoring is expected. Standard genotoxicity studies, as applied to a conventional chemical drug, are not relevant for this type of product and were hence not performed. In the carcinogenicity program, the applicant discussed why it was not possible to generate suitable experimental conditions to allow for long term analysis of the in vivo carcinogenicity potential of the test item. As clarified, after administration of GSK2959346, the VCN achieved was low and there was no long term engraftment of transduced cells in mice. To increase the likelihood of long term engraftment, alterations were made to optimise transduction conditions for murine cells and for engraftment. The impact of different transduction conditions of cells was tested in vitro and in vivo; the effect of the genetic background of mouse cells and the type of vector used were also examined. In the short term, transduction of murine cells and engraftment into mice was achieved. However, the applicant states that it was not possible to demonstrate stable, long term engraftment of transduced cells under any condition tested in these pilot studies (with the exception of cells transduced with a lentiviral vector carrying ADA cDNA). The attempted in vitro and in vivo approaches to allow for the assessment risk of clonal expansion and tumour arising from genetically modified cells, although unsuccessful, are accepted as comprehensive investigations. The study description indicates they were scientifically thought through and it is accepted that no further investigations are warranted at this time. From a non-clinical perspective, the carcinogenic potential due to insertional mutagenesis and the potential for subsequent clonal expansion cannot be determined at the time of assessment. Accordingly, the SmPC and RMP have been updated and the applicant has agreed to a long-term follow up of patients in clinical practise (15 years) and to monitoring of potential mutagenicity.

In the single dose biodistribution/toxicology study, marked atrophy in gonads of both sexes was observed. It was concluded that this was a consequence of busulfan conditioning. Germline transfer is

regarded a major concern in human gene therapy. It is noted that the risk of germline transfer associated with the administration of genetically modified human cells is considered to be low and animal testing of human cells may be difficult if not meaningful; hence non-clinical germline transmission studies of human genetically modified cells are generally not recommended. This is therefore dependent upon clinical monitoring. However, most ADA-SCID patients are treated within the first few years of life before puberty begins.

No further studies with GSK2696273 were conducted and the absence of any additional studies is adequately justified.

Finally a comprehensive environmental risk assessment (ERA) was conducted in accordance with Directive 2001/18/EC on the deliberate release into the environment. Considering the data discussed in the ERA, Strimvelis is not expected to pose a risk to the environment providing handling precautions are carried out as stated in the SmPC in order to minimise any potential risks to the environment.

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

2.3.7. Conclusion on the non-clinical aspects

The CAT considered the non-clinical data adequate for characterisation of the non-clinical profile of Strimvelis. The lack of certain studies was fully justified and there are no unresolved issues.

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

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant. The applicant has clarified that the pivotal study was initiated as an investigator-driven study in 2002, but from the time GSK took over sponsorship, this study was performed in compliance with Good Clinical Practices.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Summary of Studies Contributing Evidence of Efficacy and Safety

Study ID	AD1115611[a] Pivotal	AD1117056 Pilot 2	AD1117054 Pilot 1	AD1117064 CUP	AD1115611 LTFU	200893 NPP
Level of Evidence	Pivotal	Supportive	Supportive	Supportive	Pivotal[b]	Supportive[h]
Number of Subjects	12	2	1[c]	3	17	2
Site (Location)	HSR (Milan, Italy)	HSR (Milan, Italy)	Hadassah University Hospital (Jerusalem, Israel)	HSR (Milan, Italy)	HSR (Milan, Italy) and Hadassah University Hospital (Jerusalem, Israel)	HSR (Milan, Italy)
Critical Design Features	Phase 1/2, open-label, non-randomized, historical control, single arm	Phase 1/2, open-label	Phase 1, open-label[d]	Compassionate use program (CUP)	LTFU of pivotal study[e]	Named Patient Program (NPP)[f]
Primary Endpoint	3-year survival	Not defined[g]	Not defined[g]	NA	Survival	NA
Study Population	Pediatric patients with ADA-SCID lacking an HLA-identical sibling who had received ≥6 months PEG-ADA with demonstrated failure to PEG-ADA therapy (except in cases where PEG-ADA therapy was contraindicated or unavailable)					
Treatment Regimen	Non-myeloablative pre-conditioning with busulfan followed by gene therapy with GSK2696273, defined as transfusion of autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with a retroviral vector that encodes for the human ADA cDNA sequence					
Study Status	Complete	Complete	Complete	Complete[i]	Ongoing	Ongoing
Report data cut-off	06 Jul 2011	24 Feb 2005	25 Nov 2013[c]	08 May 2014	08 May 2014	8 wks prior to submission[h]
Source	AD1115611 CSR	AD1117056 abbreviated CSR	AD1117054 CSR	AD1117064 CSR	AD1115611 LTFU interim CSR	SAE narratives (Section 7.4 of SCS [m2.7.4])[h]
Module Location	m5.3.5.1	m5.3.5.2	m5.3.5.4	m5.3.5.4	m5.3.5.2	NA

Abbreviations: ADA-SCID = adenosine deaminase severe combined immunodeficiency; cDNA = complementary deoxyribonucleic acid; CSR = clinical study report; CUP = compassionate use program; HLA = human leukocyte antigen; HSR-TIGET = San Raffaele Telethon Institute for Gene Therapy Ospedale San Raffaele s.r.l. (formerly Fondazione Centro San Raffaele del Monte Tabor) Istituto Scientifico San Raffaele; LTFU = long-term follow-up; NA = not applicable; PEG-ADA = polyethylene glycol modified bovine adenosine deaminase; wks = weeks.

a. Pivotal study (AD1115611) initiated with HSR-TIGET and transferred to GSK upon 2010 in-licensing.
b. LTFU data from Subjects 4 to 15 (originally enrolled in the pivotal study, AD1115611) are considered as pivotal evidence of safety in this application. LTFU data from other subjects are considered supportive.
c. Data for [redacted] Years 0 to 13 were not integrated, except for the date of gene therapy used to determine duration of follow-up and survival. Data handling for Subject [redacted] is summarized in Section 1.6 of the SCE (m2.7.3) and Section 1.1.7.1 of the SCS (m2.7.4). Data for Subject [redacted] from Years 0 to 12 are not included in the integrated safety data, with the exception of the date of gene therapy which was used to determine duration of follow-up.

2.4.2. Pharmacokinetics

No dedicated PK-PD studies have been conducted as a conventional clinical pharmacology programme, including dose escalation/dose range finding, human absorption, metabolism and excretion, drug-drug interaction and special population studies is not considered feasible for GSK2696273. However, a range of clinically relevant doses were evaluated in the clinical studies and exploratory analyses of dose

correlations with treatment outcomes were conducted. Potential metabolic differences based on age, gender, or race were not assessed in dedicated studies or in the pivotal population but adverse events for these identifiable factors were collected and are described for the integrated safety population. The following parameters were monitored in the clinical studies of GSK2696273, with respect to pharmacokinetics and pharmacodynamics in accordance with the 2012 EMA Guidance on "Quality, Non-clinical and Clinical Aspects of Medicinal Products Containing Genetically Modified Cells" (EMA/CAT/GTWP/671639/2008):

- The percentage of gene modified cells in various cell lineages in the bone marrow and peripheral blood over 8 years of follow up after gene-therapy.
- Distribution of gene modified cells in bone marrow and peripheral blood over 8 years of follow up after gene therapy.
- In the pivotal study AD1115611, a long-term safety monitoring plan was implemented that included evaluation of RCR from bone marrow and peripheral blood samples archived during the 3 year follow up.
- Retroviral insertions site (RIS) analysis was conducted on 14 subjects during the Long term follow up (LTFU).
- The presence of the ADA transgene measuring ADA activity
- Modification of the systemic metabolic defect was analysed by measuring levels of purine metabolites (dAXP) in bone marrow and peripheral blood.
- Functional endpoints indicative of reconstitution of immune function following gene therapy.

No immunogenicity testing has been conducted. The applicant stated that GSK2696273 is comprised predominately of autologous-derived cells with intracellular ADA, in which an immunogenic response of any sort would be unlikely. Indirect evidence that there is no harmful reaction to the ADA-expressing cells has been observed in the GSK2696273 programme, including persistence of gene corrected cells, long-term ADA expression, and effective detoxification. While infectious vector particles could potentially remain in the drug product after transduction of patient CD34+ cells, these biodistribution studies have not show any evidence of transduction of non-target cells nor was vector DNA detected in the absence of donor cells. In the pivotal clinical study, all testing for replication competent recombinant retrovirus (RCR) in bone marrow and peripheral blood was negative.

2.4.3. Pharmacodynamics

Mechanism of action

GSK2696273 consists of an autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human adenosine deaminase complementary DNA sequence. It is intended to be administered on a single occasion since after infusion CD34+ cells migrate to the bone marrow where they engraft and give rise to cells that repopulate the haematopoietic system and act as a source of circulating blood cells producing pharmacologically active levels of intracellular ADA enzyme. Following successful engraftment in the patient, the effects of the product are intended to be life-long. The progeny of the engrafted bone marrow cells would be expected to become part of the transient population in all tissues and/or contribute to resident haematopoietic cells in specific tissues (i.e., lymphoid tissues, spleen, lymph nodes, mucosa-associated lymphoid tissue, thymus, Kupffer cells in liver, alveolar macrophages and microglia in the central nervous system). However, there is no expectation that GSK2696273 itself would cross the blood brain

barrier or glomerulus or be exposed to the liver cytochrome family of enzymes. The lifespan of this bone marrow cell pool is not determined but is assumed to be long-lived, as circulating ADA-transgene expressing blood cells continue to be produced long-term. The ADA protein expression occurs in transduced cells and is not expected to be found in extracellular spaces. Elimination of ADA-expressing cells in the circulation is expected to be via the same mechanism as their non-transduced counterparts. Hence, the primary mechanism of action of GSK2696273 is via genetic modification of cells and restoration of production/activity of ADA. However, the presence of unmodified cells in the product is necessary to support broad hematopoietic system reconstitution after gene therapy. The mechanism of action of GSK2696273 gene therapy was evaluated by means of measuring engraftment of gene modified cells per VCN in bone marrow and peripheral blood cells (erythroid, granulocyte, and lymphocyte lineages), ADA activity in bone marrow and peripheral blood lymphocytes, and correction of the systemic metabolic defect as measured by dAXP levels in RBCs from bone marrow and peripheral blood. The results of these evaluations were studied in both the pivotal population and the integrated population, as discussed in the section on Clinical efficacy.

Primary and Secondary pharmacology

Presence of gene modified cells

During the development of GSK2696273, viral copy number (VCN) has been used to track the prevalence of gene marked cells in peripheral blood and bone marrow following gene therapy using a quantitative real-time polymerase chain reaction (PCR) assay to assess genomic DNA for vector specific sequences relative to a human housekeeping gene. VCN data is commonly reported as percent gene modified cells. The assay reveals a ratio of the number of copies of vector relative to the number of copies of the housekeeping gene. As some cells in the population are transduced with multiple copies of vector, the translation may represent an overestimate of the actual percent gene modified cells. VCN analysis has been applied to CD34+ bone marrow cell fractions that make up the GSK2696273 product, making it possible to study the extent to which gene modified stem cells divide normally and give rise to the various cell lines detectable in peripheral blood.

The analysis provided is therefore a measure of percent genetically modified cells, mainly peripheral blood granulocytes, as an example of a cell lineage that is not normally impacted by ADA-SCID, and several lymphocyte populations that are profoundly negatively impacted by ADA-SCID. In both the Pivotal and the Integrated populations, median CD15+ granulocyte proportions of gene marked cells in peripheral blood were typically >0.5% up to approximately 4.5% during follow-up, see table below.

Summary Statistics of Log-Transformed Genetically Modified CD3+ and CD15+ Cells in Peripheral Venous Whole Blood (Pivotal and Integrated Populations)

Cell marker Visit	Pivotal Population (N=12)			Integrated Population (N=18)		
	n	Mean (95% CI), %	Median (min-max), %	n	Mean (95% CI), %	Median (min-max), %
CD3+						
Month 6	11	26.702 (11.347, 62.836)	37.150 (3.40-101.00)	16	22.867 (11.280, 46.358)	34.150 (1.39-101.00)
Year 1	10	58.461 (39.248, 87.078)	73.100 (20.30-100.00)	14	63.723 (47.408, 85.652)	76.450 (20.30-101.00)
Year 2	11	67.725 (53.017, 86.514)	75.050 (35.60-100.00)	15	65.877 (55.203, 78.614)	72.550 (35.60-100.00)
Year 3	11	63.763 (55.374, 73.424)	67.050 (39.05-81.95)	14	67.195 (58.644, 76.993)	68.390 (39.05-101.00)
Year 4	8	77.196 (61.248, 97.296)	81.688 (42.52-100.00)	10	80.838 (66.957, 97.598)	85.073 (42.52-101.00)
Year 5	9	88.764 (78.085, 100.904)	91.700 (59.06-101.00)	10	82.236 (66.914, 101.067)	90.685 (41.35-101.00)
Year 6	5	94.312 (83.030, 107.128)	98.150 (79.06-101.00)	6	77.483 (46.326, 129.594)	96.210 (29.00-101.00)
Year 7	6	79.634 (60.192, 105.355)	85.315 (58.54-101.00)	6	79.634 (60.192, 105.355)	85.315 (58.54-101.00)
Year 8	2	93.922	94.170 (87.34-101.00)	3	72.203 (22.957, 227.087)	87.340 (42.67-101.00)
CD15+						
Month 6	11	0.406 (0.116, 1.419)	0.500 (0.03-6.50)	16	0.413 (0.150, 1.140)	0.610 (0.00-6.50)
Year 1	10	0.645 (0.238, 1.749)	0.650 (0.05-5.20)	13	0.848 (0.367, 1.962)	0.800 (0.05-7.40)
Year 2	11	0.553 (0.226, 1.352)	0.400 (0.10-7.50)	14	0.644 (0.278, 1.496)	0.450 (0.10- 7.50)
Year 3	11	0.744 (0.319, 1.735)	0.600 (0.010-6.67)	14	0.689 (0.251, 1.892)	0.750 (0.01-6.67)
Year 4	8	1.005 (0.352, 2.869)	1.005 (0.16-5.79)	10	0.796 (0.224, 2.835)	1.005 (0.02-5.79)
Year 5	9	1.186 (0.413, 3.410)	1.170 (0.19-10.90)	10	1.245 (0.490, 3.164)	1.190 (0.19-10.90)
Year 6	5	0.676 (0.146, 3.122)	0.910 (0.16-2.60)	6	0.979 (0.219, 4.386)	1.300 (0.16-6.26)
Year 7	6	2.040 (0.653, 6.373)	2.360 (0.60-6.85)	6	2.040 (0.653, 6.373)	2.360 (0.60-6.85)
Year 8	2	3.096	4.485 (1.24-7.73)	3	3.213 (0.329, 31.366)	3.460 (1.24-7.73)

Abbreviations: CI = confidence interval; max = maximum; min = minimum.
Note: The 95% CIs are not provided if n<3. An imputation was applied to any value where the observed value=0 in order to log transform the data. Data collected as >100 are imputed to 101.

Following the gene therapy, gene modified cells appeared in the circulation quickly (6 months), and the numbers remained stable for the duration of >5 years. As granulocytes are short-lived and do not have a survival advantage following gene transduction, the percentage of gene marked CD15+ granulocyte is a surrogate marker of the proportion of gene modified stem cells in bone marrow. The proportion of gene modified CD34+ cells in bone marrow is consistent with the results for CD15+ cells in peripheral blood. Bone marrow stem cells carrying the ADA transgene were able to support lymphocyte development in the periphery. In both, the pivotal and integrated populations, gene marking in CD34+ T cells was approximately 70% or higher from Year 1 and onwards of follow-up, see table above. This high level of gene marking compared with other lineages is explained by the selective advantage in lymphocytes for ADA expressing cells. The data show there was no loss of gene marking in engrafted bone marrow and peripheral cells over 8 years of follow-up after receiving GSK2696273 treatment. Similarly to granulocytes, a relatively low proportion of gene modified cells was shown in the erythroid lineage, consistent with lack of survival advantage for this blood lineage. This is relevant because ADA activity in RBCs is a commonly used marker for disease severity, but is less informative for subjects who have received gene therapy for which the treatment effect is more derived from lymphocyte ADA expression.

An exploratory responder analysis was conducted to determine the proportion of subjects at each time point with bone marrow engraftment and peripheral circulation of gene modified cells. The definition of a responder was developed based on previous experience with other gene therapy studies in the literature. Both of the following criteria had to be met to be considered a responder:

- Presence of ≥0.1% gene marked cells in bone marrow for both CD15+ granulocytes and erythroid cells (assessed by glycophorin A) and at least one of the CD3+ T cell, CD19+ B cell, or CD56+ NK subsets; and
- Presence of ≥10% gene marked cells in peripheral blood CD3+ T cells and CD19+ B cells.

At Year 1, among subjects with available data in the pivotal population, most were VCN responders: 9 of 11 subjects (82%) met response criteria, 2 subjects (18%) did not. This trend continued throughout follow-up to Year 8, with 82% to 100% of subjects considered responders at each time point. The pivotal population data were supported by results in the integrated population. Among subjects with available data in the integrated population, most were responders at Year 1: 11 of 15 subjects (73%). Similar to the pivotal population, this trend continued throughout follow-up to Year 8, with 79% to 100% of subjects per time point considered as responders.

Lymphocyte ADA Activity

In the pivotal population, lymphocyte ADA activity by Year 1 showed increased levels relative to baseline, which were maintained for the duration of follow-up to Year 8 ADA activity was similar in the integrated population. All subjects treated in the pivotal study (AD1115611) and the compassionate use programme (CUP) (AD1117064) were receiving PEG-ADA prior to gene therapy. Per protocol, PEG-ADA was discontinued approximately 2 to 3 weeks prior to gene therapy. Possible carryover effects of PEG-ADA exposure on early ADA activity were anticipated. Intracellular lymphocyte ADA activity was low at baseline, consistent with the disease. Following gene therapy, there was a marked increase in ADA activity in lymphocytes that was maintained for the duration of follow-up. The same pattern of activity was shown for the integrated population. Minor levels of ADA activity were evident in other cell lineages, e.g. erythrocytes.

An exploratory responder analysis was conducted to determine the proportion of subjects at each time point with lymphocyte ADA activity ≥210 nmol/h/mg. This value represents 10% of the mean value for healthy subjects and is considered clinically relevant as it is a threshold for minimum normal activity and corresponds to a minimum ADA activity level reported to result in normal function in the literature. From Year 2 onwards, the majority of subjects with available data in the pivotal population were ADA responders: 5 of 9 subjects (56%) met response criteria in Year 2. This trend was maintained in follow-up to Year 8, with 75% to 100% of subjects considered responders at all time points except Year 4, which included data for only 5 subjects among whom 1 (20%) was a responder. The pivotal population data were supported by results in the integrated population (see table below). Among subjects with available data in the integrated population, most were ADA responders from Year 2 onwards: 8 of 12 subjects (67%) met response criteria at Year 2. Similar to the Pivotal population, this trend was maintained throughout follow-up to Year 8, with 67% to 100% of subjects per time point considered as responders at each time point except Year 4, for which only 6 subjects had available data among whom 2 (33%) were responders.

Summary Statistics of Lymphocyte ADA Activity (Pivotal and Integrated Populations)

Visit	Pivotal Population (N=12)			Integrated Population (N=18)		
	n	Mean (95% CI), nmol/h/mg	Median (min-max), nmol/h/mg	n	Mean (95% CI), nmol/h/mg	Median (min-max), nmol/h/mg
Baseline	5	71.230 (40.191, 102.269)	80.550 (30.53-92.29)	7	57.450 (28.620, 86.280)	65.000 (21.00-92.29)
Month 6	5	295.584 (-174.572, 765.740)	87.720 (2.20-869.00)	6	268.535 (-93.620, 630.690)	110.505 (2.20-869.00
Year 1	10	561.651 (94.810, 1028.492)	181.245 (42.11-1678.17)	14	479.665 (149.111, 810.219)	161.975 (42.11-1678.17)
Year 2	9	360.056 (157.730, 562.381)	252.770 (88.38-843.24)	12	400.833 (232.204, 569.461)	343.000 (88.38-843.24)
Year 3	11	454.272 (295.079, 613.465)	464.940 (122.11-932.55)	13	459.834 (328.100, 591.568)	464.940 (122.11-932.55)
Year 4	5	191.586 (65.851, 317.321)	176.040 (96.44-360.11)	6	221.083 (99.494, 342.673)	183.715 (96.44-368.57)
Year 5	9	376.379 (261.815, 490.943)	395.270 (120.00-567.55)	10	356.957 (247.254, 466.660)	386.485 (120.00-567.55)
Year 6	4	381.353 (-68.469, 831.174)	267.905 (189.89-799.71)	5	372.524 (67.558, 677.490)	301.950 (189.89-799.71)
Year 7	6	695.772 (186.511, 1205.032)	539.490 (263.51-1604.76)	6	695.772 (186.511, 1205.032)	539.490 (263.51-1604.76)
Year 8	2	291.105	291.105 (254.56-327.65)	3	249.387 (48.236, 450.537)	254.560 (165.95-327.65)

Abbreviations: CI = confidence interval; max = maximum; min = minimum.
Note: The 95% CI are not provided if n<3.

Of note, all patients in the pivotal study and CUP had received PEG–ADA prior to gene therapy and therefore some carryover would be expected. Although ADA activity appears to be evident from year 2, it is not entirely clear what the cause of poor response is in Year 4 where only 1/5 (20%) of patients was classed as a responder. It is also not clear whether there were any clinical consequences as a result of the poor response seen in Year 4. However, the applicant also highlighted the arbitrary nature of the responder level for lymphocyte ADA, which was established in the literature. Nevertheless, these Year 4 non-responders became responders in Year 5, showed adequate metabolic detoxification and were considered treatment successes, remaining clinically well, which is reassuring. Importantly, no significant clinical consequences with respect to severe infections were noted.

Purine Metabolites

Modification of the systemic metabolic defect was analysed by measuring levels of purine metabolites in bone marrow and peripheral blood, where levels of dAXP in RBCs were correlated with severity of disease. Adequate systemic metabolic detoxification was classified as levels of purine metabolites (dAXP=dAMP+dADP+dATP) in RBC <100 nmol/mL. Typically, most patients with ADA-SCID have RBC levels of dAXP >350 nmol/mL, whereas most patients with delayed, late-onset, or partial phenotypes have RBC levels of dAXP <300 nmol/mL. Patients with ADA-SCID who have received a SCT or gene therapy have been reported as having dATP levels <100 nmol/mL. Levels of dAXP were measured in RBCs from bone marrow and peripheral blood. Analysis of the pivotal and integrated populations showed no appreciable reduction in dAXP levels in RBCs from bone marrow or peripheral venous blood for up to 3 months following gene therapy, presumably due to low baseline values that may have resulted from carryover effect of prior PEG-ADA use (discontinued per protocol approximately 2 to 3 weeks prior to gene therapy) as well as the low number of mature cells in the periphery that arose from gene-modified stem cells. Post-baseline mean and median dAXP were below pathological levels (100 nmol/mL) and this trend was maintained for the duration of follow-up, see table below. All available data are shown for dAXP levels in bone marrow-derived RBCs, and do not indicate decrease from baseline. These data show restored adenosine and adenosine metabolite clearance, evidenced in peripheral RBCs, by 6 months post-gene therapy.

Summary Statistics for Log-Transformed dAXP Levels in RBCs (Pivotal and Integrated Populations)

Source Visit	Pivotal Population (N=12)			Integrated Population (N=18)		
	n	Geo mean (95%CI), nmol/mL	Median (min-max), nmol/mL	n	Geo mean (95%CI), nmol/mL	Median (min-max), nmol/mL
Bone marrow						
Baseline	7	19.7 (1.9-209.1)	9.0 (0-872)	10	20.2 (3.4, 121.0)	16.0 (0-872)
Year 1	10	41.4 (21.3-80.6)	53.0 (11-161)	13	51.9 (29.7, 90.7)	74.0 (11-161)
Year 2	10	18.9 (6.5-54.8)	32.5 (0-75)	12	22.4 (9.2, 54.6)	39.0 (0-75)
Year 3	10	17.8 (7.1-44.8)	28.5 (0-64)	13	20.8 (43.3, 184.35)	29.0 (0-68)
Year 5	8	31.3 (9.2-106.8)	54.0 (0-81)	9	31.1 (89.5, 236.62)	41.0 (0-81)
Year 8	2	23.5	23.5 (23-24)	3	26.0 (16.7, 40.7)	24.0 (23-32)
Venous blood						
Baseline	11	11.6 (3.2, 42.7)	10.0 (0-358)	12	15.2 (4.1, 56.0)	12.0 (0-358)
Month 6	11	55.7 (26.6, 116.6)	66.0 (4-232)	16	58.3 (35.1, 97.0)	66.5 (4-232)
Year 1	11	48.7 (33.8, 70.3)	54.0 (17-100)	14	55.7 (39.2, 79.2)	58.0 (17-147)
Year 2	10	25.2 (13.1, 48.3)	29.5 (5-69)	13	28.7 (15.8, 52.0)	30.0 (5-112)
Year 3	11	22.8 (10.4, 50.0)	28.0 (0-69)	14	24.5 (12.5, 47.8)	29.0 (0-113)
Year 4	8	21.9 (6.7, 72.0)	33.5 (0-73)	10	23.8 (8.9, 63.4)	33.5 (0-100)
Year 5	9	29.5 (9.5, 90.9)	28.0 (0-114)	10	28.9 (10.7, 77.7)	26.0 (0-114)
Year 6	5	25.8 (2.5, 268.1)	50.0 (0-104)	6	22.4 (3.7, 136.4)	39.0 (0-104)
Year 7	6	31.0 (14.6, 65.8)	40.0 (12-64)	6	31.0 (14.6, 65.8)	40.0 (12-64)
Year 8	2	29.1	32.5 (18-47)	3	25.2 (6.6, 96.3)	19.0 (18-47)

Abbreviations: CI = confidence interval; Geo mean = geometric mean; max = maximum; min = minimum.
Note: The 95% CIs are not provided if n<3. An imputation was applied to any value where the observed value=0 in order to log transform the data. Venous blood refers to peripheral whole blood.

A responder analysis was conducted at each time point to determine the proportion of subjects who had adequate systemic metabolite detoxification, assessed as dAXP levels in peripheral blood RBCs. Correction of the systemic metabolic defect of ADA deficiency was demonstrated by analysis of bone marrow from Year 2 onwards in the Pivotal Population, with 100% of subjects considered as responders at Year 2 onwards, see table below.

Responder Analysis of Log-Transformed dAXP Levels in RBCs (Pivotal and Integrated Populations)

Source Visit	Pivotal Population (N=12)				Integrated Population (N=18)			
	n	n (%) Responders	95% CI	P-value	n	n (%) Responders	95% CI	P-value
Bone marrow								
Year 1	10	9 (90)	(55, 100)	<.001	13	10 (77)	(46, 95)	<.001
Year 2	10	10 (100)	(69, 100)	<.001	12	12 (100)	(74, 100)	<.001
Year 3	10	10 (100)	(69, 100)	<.001	13	13 (100)	(75, 100)	<.001
Year 5	8	8 (100)	(63, 100)	<.001	9	9 (100)	(66, 100)	<.001
Year 8	2	2 (100)		<.001	3	3 (100)	(29, 100)	<.001
Venous blood								
Year 1	11	10 (91)	(59, 100)	<.001	14	11 (79)	(49, 95)	<.001
Year 2	10	10 (100)	(69, 100)	<.001	13	12 (92)	(64, 100)	<.001
Year 3	11	11 (100)	(72, 100)	<.001	14	13 (93)	(66, 100)	<.001
Year 4	8	8 (100)	(63, 100)	<.001	10	9 (90)	(55, 100)	<.001
Year 5	9	7 (78)	(40, 97)	<.001	10	8 (90)	(44, 97)	<.001
Year 6	5	4 (80)	(28, 99)	<.001	6	5 (83)	(36, 100)	<.001
Year 7	6	6 (100)	(54, 100)	<.001	6	6 (100)	(54, 100)	<.001
Year 8	2	2 (100)		<.001	3	3 (100)	(29, 100)	<.001

Abbreviations: CI = confidence interval.
Note: The 95% CIs are not provided if n<3. An imputation was applied to any value where the observed value=0 in order to log transform the data. Note: Adequate systemic metabolic detoxification classified as levels of purine metabolites dAXP=dAMP+dADP+dATP) in RBCs <100 nmol/mL. The p-value is one-sided for treatment effect versus a reference value of 10%

As with ADA activity, modification of the systemic metabolic defect is evident from year 2 with 100% response. Although no value is available for bone marrow, the value at Year 4 for peripheral blood still shows 100% response in contrast to the ADA activity which was noted in only 20%. This inconsistency was clarified by the applicant who highlighted the fact that the cut-off values for responder analysis for metabolic detoxification and ADA were based on the clinical literature, as well as the fact that the non-responders with respect to lymphocyte ADA did show ADA levels close to the response threshold and did become responders at Year 5 as well as showing clinical response. A comparison of the Year 4 ADA enzyme activity to the corresponding dAXP levels, appears to suggest that ADA enzyme activity at Year 4 point may be sufficient for metabolic detoxification, as the ADA responder subjects and all of the ADA enzyme non-responders showed adequate metabolic detoxification based on the dAXP levels.

2.4.4. Discussion on clinical pharmacology

No dedicated PK-PD studies have been conducted because the conventional clinical pharmacology programme including dose escalation/dose range finding, human absorption, metabolism and excretion, drug-drug interaction and special population studies is not considered feasible for the gene therapy product GSK2696273. However, a range of clinically relevant doses were evaluated in the clinical studies, and exploratory analyses of correlations of dose with treatment outcomes were conducted. This is considered appropriate in the circumstance for this particular class of product. Various parameters relating to pharmacology of the product were monitored in the clinical studies of GSK2696273, with respect to pharmacokinetics and pharmacodynamics. This is considered to be in accordance with the 2012 EMA Guidance on "Quality, Non-clinical and Clinical Aspects of Medicinal Products Containing Genetically Modified Cells" [EMA/CAT/GTWP/671639/2008]. The parameters chosen by the applicant appear to be both appropriate and consistent with the above guideline.

Moreover, the parameters studied to evaluate the mechanism of action of the product would appear to be consistent with each other and provide evidence of engraftment of genetically modified cells, production of the deficient enzyme to therapeutic levels as well as showing functionality of the enzyme by the modification of the systemic metabolic defect by achieving below toxic levels of the purine metabolites, which was a key secondary efficacy endpoint. Regarding the presence of genetically modified cells in the bone marrow compartment, it is noted that despite the low number of CD34- transduced cells, the proportion of genetically modified lymphocytes is very high, both in bone marrow and peripheral blood. Indirectly, the high proportion of CD3+ T lymphocytes carrying the transgene points to local expansion of this population outside the thymus.

2.4.5. Conclusions on clinical pharmacology

The CAT considered the clinical pharmacology development programme to be adequate for this type of an advance therapy medicinal product. All outstanding issues raised during the procedure have been satisfactorily resolved.

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

2.5. Clinical efficacy

2.5.1. Dose response study

No formal dose ranging studies were conducted in the clinical development of GSK2696273. The recommended dose of this product is 2 to 20x106 CD34+ cells/kg, which is based on the paediatric haematopoietic transplant clinical recovery data. The lower dose limit is considered appropriate as it is supported by literature data. The recommended dose is furthermore supported by data from one subject who received <2x106 CD34+ cells/kg and had an unsuccessful response to the gene therapy. The maximum dose was essentially defined by practical limitations of bone marrow harvesting and the manufacturing process.

2.5.2. Main studies

Title of the pivotal study AD1115611: Open-label, prospective, sequential study in children with SCID due to ADA deficiency who lacked a healthy HLA-identical sibling.

The Committee noted that the applicant mentioned that they acquired management of the programme of the pivotal study in 2010 and has not been able to demonstrate full GCP compliance for all elements of pilot studies 1 and 2. Consequently, in agreement with the regulatory feedback obtained during the pre-MAA meeting (July 2014), the efficacy conclusions are drawn from the 12 subjects (Pivotal population) treated within the AD1115611 pivotal study (including their LTFU data).

The efficacy data presented in the dossier are different from the data presented in the CSR for AD1115611. In the dossier, but not in the CSR, data are censored from subjects who received ≥3 continuous months of PEG-ADA or allogeneic SCT occurring post- GSK2696273 gene therapy. This decision was made following the review of the CSR, to allow differentiation of any treatment effect from the effects of the interventional treatment, and the data are considered more meaningful than the analyses presented in the CSR. It should be noted that, with the exception of survival data, all efficacy data from the integrated 18 subjects have been included in the summary of clinical efficacy of the submitted dossier as supportive data only. In addition, the integrated population of all 18 subjects was used for the primary endpoint of survival. The Committee agrees with the view that analysis of the

censored data would be more clinically meaningful since early trials in gene therapy were confounded by the concomitant use of PEG-ADA. The Committee also considers censoring of the data as appropriate. The applicant has chosen to present data for both the pivotal as well as the integrated population for this analysis. Available efficacy data for subjects treated with GSK2696273 are reported for the pivotal population (N=12) and the integrated population (N=18):

- **Pivotal population**: included subject data from 0-3 years and LTFU, for only the 12 subjects originally treated within the pivotal study (AD1115611). The Pivotal population is the primary population for efficacy analysis in the summary of clinical efficacy.
- **Integrated population**: included subject data from 0-3 years and LTFU for subjects originally treated in the pivotal study (AD1115611), Pilot Study 2 (AD1117056), Pilot Study 1 (AD1117054; survival data only), and the CUP (AD1117064).

Methods

Study Participants

Subjects aged <18 years suffering from SCID with ADA deficiency, as assessed by ADA enzymatic activity and/or genetic analysis, and for whom an HLA-identical healthy sibling was not available as suitable bone marrow donor, were enrolled. In addition, subjects had to fulfil at least 1 of the following criteria:

- Subjects who had received enzyme replacement therapy (PEG-ADA) for at least 6 months before enrolment and displayed at least 2 of the following immune parameter alterations:
- Absolute lymphopenia (<1500 cells/μL)
- Absolute T lymphopenia (<1000 cells/μL)
- Requirement for intravenous immunoglobulin (IVIG) infusion
- Deficit of serum immunoglobulins (IgM or IgA or subclasses of IgG) or lack of antibody response to vaccination
- Subjects who had received enzyme replacement therapy (PEG-ADA), and in whom the drug had been discontinued due to intolerance, allergy, or autoimmune manifestations
- Subjects for whom enzyme replacement therapy (PEG-ADA) was not a life-long therapeutic option (e.g., from countries in which the drug is not available)

Treatments

Pre-Treatment

Stem cell back-up: at least 3 weeks before gene therapy, subjects underwent bone marrow harvest, to collect stem/progenitor cells as rescue therapy available for future infusion.

In case of the availability of stem cells from different sources (bone marrow or umbilical cord blood, or Mobilised peripheral blood) collected before subject enrolment, these may have been considered a valid back-up. The back-up was to contain at least 1 x 10^6 CD34+ cells/kg and was frozen and stored un-manipulated in liquid nitrogen. The back-up was to be used in case of transplant failure or prolonged bone marrow aplasia.

Treatment

The medicinal product consisted of autologous stem/progenitor cells transduced ex vivo with the GIADAl retroviral vector encoding ADA cDNA. Enrolled subjects were hospitalised during the treatment phase in the Paediatric Clinical Research Unit HSR-TIGET. (For clarification, 1 subject in the pilot study was treated at Hadassah University [Jerusalem, Israel; this subject was invited to be enrolled into the long-term safety follow-up phase as part of Protocol Amendment No. 5). In the treatment phase 4 steps were planned:

1. Harvest of bone marrow: Bone marrow collection of 20–25 mL/kg subject weight was usually expected to give the optimal target of CD34+ cell count before manipulation (10–20 x 106 CD34+ cells/kg) to achieve an optimal dose of 5–10 x 106 CD34+ cells/kg. A minimum amount of bone marrow CD34+ cells (3–4 x 106 CD34+ cells/kg) was recommended to reach a minimum dose of 2 x 106 CD34+ cells/kg. On the other hand, in order not to exceed the highest recommended reinfusion dose of 20 x 106 CD34+ cells/kg, less than 30–40 x 106 CD34+ cells/kg were to be collected. Haemoglobin values <7.0 g/dL were corrected by irradiated and filtered RBC transfusion.

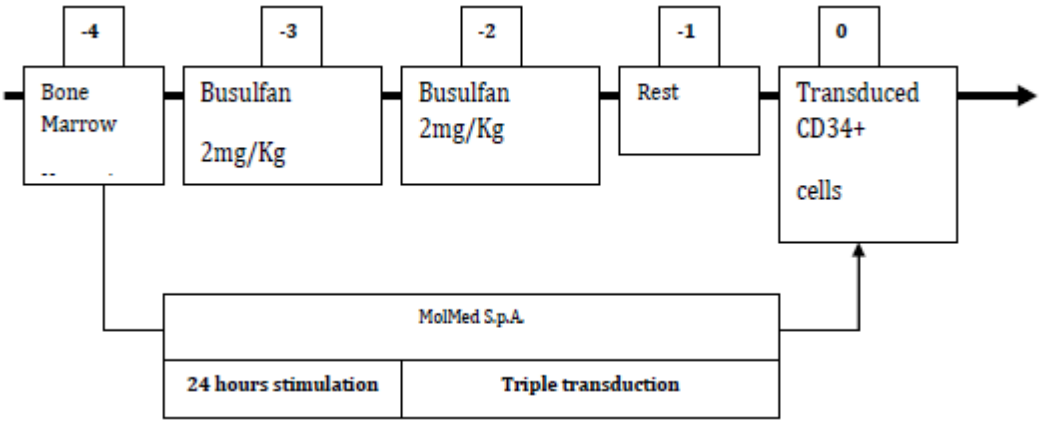
2. Cell manipulation and gene transfer

3. Busulfan conditioning: The conditioning with i.v. busulfan was at non-myeloablative doses, scheduled at 2 mg/kg/day (divided into 4 doses of 0.5 mg/kg each) on Days -3 and -2 (total final dose 4 mg/kg). If the administration of i.v. busulfan was not possible (e.g., occlusion of the CVC), busulfan was to be administered orally (4 doses of 0.6 mg/kg).

4. Infusion: The final medicinal product consisted of a cell suspension at the concentration of 4–10 x 106/mL, in a sterile syringe of the suitable volume. The exact dose of CD34+ cells/kg received by each subject was dependent upon the number of CD34+ cells available following bone marrow extraction, their growth during the manufacturing process, and the % CD34+ cells present at the end of the transduction procedure.

A treatment administration flow-chart is presented in the figure below.

Treatment Administration Flow-Chart



Objectives

- Evaluation of the safety and the clinical efficacy of gene therapy, in the absence of enzyme replacement therapy.
- Evaluation of biological activity (engraftment, ADA expression) of ADA-transducedCD34+ cells and their haematopoietic progeny.

- Evaluation of immunological reconstitution and purine metabolism after gene therapy.
- Evaluate the change in quality of life over time in ADA-SCID subjects following treatment with GSK2696273. This objective applied to the long-term follow-up (4 to 8 years after gene therapy) only; therefore, these results are not presented.

Outcomes/endpoints

Primary efficacy endpoint: Survival at 3 years post-gene therapy.

Secondary efficacy endpoints (in hierarchical order):

Key endpoints

- Change in the rate of severe infections (defined as infections requiring hospitalisation or prolonging hospitalisation).
- One-year change in T-lymphocyte counts (cells/μL).
- One-year modification of the "systemic" metabolic defect, analysed by levels of purine metabolites in RBC.

Other endpoints

- One-year change in the proliferative response to polyclonal stimuli.
- One-year change in thymic activity (T-cell receptor excision circles; TREC).
- Presence of genetically modified cells in the bone marrow compartment and presence of ≥10% genetically modified cells in peripheral blood lymphocytes.
- Lymphocyte ADA enzyme activity.
- One-year change in lymphocyte counts (cells/μL).
- Recovery of physical growth.
- Need for reintroduction of PEG-ADA (in subjects previously treated with PEGADA).
- Antibody response to vaccination.

The first 3 secondary endpoints listed in hierarchical/descending order of clinical importance were considered key secondary endpoints. Supplementary endpoints associated with the 2nd and 3rd key secondary endpoints (T-lymphocyte counts and modification of "systemic" metabolic defect) were also analysed for the Intent-to-Treat (ITT) and Per Protocol (PP) Populations. The primary endpoint of survival and the secondary endpoints are appropriate for this population of patients and have been agreed with the CAT/CHMP who recommended changing the original proposed primary endpoint of evaluating survival by death due to disease to death from any cause. The CAT/CHMP also recommended that all secondary efficacy endpoints that were to be assessed at 1 year were extended to 3 years. Therefore, all efficacy endpoints were evaluated at 1, 2 and 3 years.

Sample size

It was planned to treat at least 10 and at most 14 subjects. According to Protocol Amendment 4 and following consultation with the CAT/CHMP, the primary efficacy endpoint was defined to be survival at 3 years. Assuming a 50% reference survival at 3 years for matched unrelated donor (MUD) transplant of bone marrow (H. Gaspar from European Group for Blood and Marrow Transplantation meeting 2008, personal communication), which corresponds to a constant hazard rate (λ) of 0.231, a sample size of

12 subjects provides approximately 90% power to detect a 35% advantage (i.e., 85% survival, or α=0.054) in survival on gene therapy with GSK2696273. Simulation studies were performed to estimate the sample size assuming an exponential model with uniform censoring process in (1, 7.3 years), one-sample Log-Rank test was used with one-sided type I error, α =0.05, to compare survival of the study subjects under the null hypothesis (H0: λ =0.231) versus alternate hypothesis (H1: λ <0.054) with the reference survival. Since no fatal events were observed in the study, the Log Rank test was not applied for any further analyses. Early stopping statistical rules were defined to monitor the accumulating evidence to evaluate whether the effect of gene therapy was likely to be inferior to the lower boundary of the reference treatment, in which case the study was to be stopped. Since no subjects died during the 3 years of follow-up after gene therapy and the study continued to the end as planned, no further discussion or analysis is presented.

Randomisation

This was an open-label, single-arm study; therefore, no randomisation procedure was used.

Blinding (masking)

This was an open-label trial.

Statistical methods

The ITT Population included all subjects who were treated with gene therapy and had at least 1 post-therapy evaluation during the 0–3 year follow-up. Data collected after the 3- year visit were excluded from the analysis. The ITT Population was the primary efficacy analysis population. The PP Population included all subjects in the ITT Population who did not violate the protocol. The following deviations were considered major protocol violations:

- Any subject who needed the reintroduction of PEG-ADA.
- Any subject taking chronic steroids or major immunosuppressants (>3 months).
- Any subject who underwent allogenic BMT.

Depending upon when the protocol violation occurred, subject data prior to the violation were included in the PP analysis, but any data after that time point or visit were censored or excluded from the PP analysis. Analyses of the primary efficacy endpoint (survival at 3 years), and first 3 secondary efficacy endpoints (rate of severe infections, T-lymphocyte counts and purine metabolites in RBC) were repeated for the PP Population. Although all secondary endpoints were listed in hierarchical order of clinical importance, the first 3 secondary efficacy endpoints (rate of severe infections, T-lymphocyte counts and modification of "systemic" metabolic defect) were considered key secondary endpoints for the evaluation of efficacy in this study. Therefore, both ITT analysis and PP analysis were performed for the primary endpoint and the 3 key secondary endpoints to assess consistency of results from the 2 analyses and to evaluate the impact of protocol violations, if any. Supplementary endpoints associated with key secondary endpoints, T-lymphocyte counts and modification of "systemic" metabolic defect, were also analysed for both ITT and PP populations. No analysis on the PP Population was performed for the other secondary endpoints. The ITT Population was the analysis population for clinical safety evaluation.

Results

Participant flow

Twelve subjects were enrolled in the study. One subject took part in the baseline/screening phase twice and was not treated on the first occasion due to contamination of the drug product. The duration

of the Pre-Treatment Phase was on average 5.7 months (range: 10 days to 1.1 years). All subjects completed Year 1 assessments, 1 subject was withdrawn at 2.3 years, and all 11 remaining subjects completed Year 3 assessments. Subject disposition is summarised in table below.

Summary of Subject Disposition (ITT Population)

Number of subjects	Total
Number of subjects enrolled, N:	12
Number of subjects included in Intent-to-Treat population, n (%):	12 (100)
Number of subjects included in Per Protocol population, n (%):	12 (100)[a]
Number of subjects completed as planned, n (%):	11 (92)
Number of subjects withdrawn (any reason), n (%):	1 (8)
Number of subjects withdrawn for serious adverse events, n (%):	0
Number of subjects withdrawn for adverse events, n (%):	0
Primary reasons for subject withdrawal, n (%)	
Other – Investigator discretion	1 (8)

a. Subject [redacted] was excluded from the Per Protocol analyses from 4.6 months and Subject [redacted] from 2.1 years after gene therapy due to protocol deviations. (N=12 at baseline, N=11 at Year 1, N=10 at Year 2 and Year 3.)

One subject required PEG-ADA reintroduction and corticosteroid therapy approximately 5 months after gene therapy due to SAEs of neutropenia and autoimmune thrombocytopenia. This subject was withdrawn from the study approximately 2.3 years after gene therapy and is not a participant in long-term follow-up. The primary reason for withdrawal was recorded as investigator discretion: the subject was a candidate for allogeneic transplant and was moved to another clinical centre. This subject received a sibling-matched BMT from a relative that had not been available at the time of gene therapy treatment.

Recruitment

The pivotal study (AD1115611) was an open-label, prospective, single-center phase 1/2 study conducted at the HSR-TIGET in Milan, Italy. In this study, 12 subjects were enrolled starting in June 2002 to July 2011, then followed for 3 years post-treatment.

Conduct of the study

The original protocol, dated 26-Apr-2000, was amended 7 times. Amendments 1 to 4 were implemented before the transfer of sponsorship to GSK. Amendments 1 and 2 were produced before the start of the study; other amendments were implemented during the study. Key changes are summarised in the table below.

Summary of Protocol Amendments for Study AD1115611

Amendment No.	Date	Summary of changes
Amendment 01	09-Feb-2001	- Conditioning therapy was added to the protocol (Section 7C5 and Section 7C6). - Extension of eligibility criteria and change in supportive therapy (Section 7C5): "2: All patients included in this protocol will receive PEG-ADA enzyme therapy for at least 6 months before starting gene therapy, except in case of intolerance, allergy or autoimmune reaction to the medication or in case enzyme replacement therapy was not available or who could not continue the treatment for financial reasons (e.g., patients from other countries)."
Amendment 02	03-Oct-2002	The informed consent form was updated with information on safety.
Amendment 03	18-Apr-2007	In previous versions of the protocol, treatment involved ADA gene transfer into haematopoietic stem/progenitor cells and/or peripheral blood lymphocytes. In this amendment, this was changed such that treatment was by ADA gene transfer into haematopoietic stem/progenitor cells only. In addition, timing, statistical design, endpoints, promoter, principal investigator, financial sponsor, safety information in the informed consent form, and sample size were updated.
Amendment 04	08-Jul-2008	Statistical section was revised based on European Medicines Agency Protocol assistance. The event considered for the primary endpoint was changed from 'death related to disease' to 'death' and the reference survival rate was based on reported data for subjects receiving a bone marrow transplant from a matched unrelated donor, rather than subjects receiving enzyme replacement therapy. The hierarchical order of the secondary endpoints was partially changed (systemic metabolic defect was moved).
Amendment 05	09-May-2011	In October 2010, GSK R&D obtained a licence to develop the ADA-SCID treatment covered by this report. The purpose of this protocol amendment was to formally register GSK as the new sponsor for the clinical trial and align data management and SAE reporting methods with company processes. Additionally, and in line with regulatory guidance on the long-term follow-up of subjects treated with gene therapy, the total population of subjects covered by the protocol was extended to include in long-term follow-up (post 3 years) 3 subjects treated in 2 pilot studies and 3 subjects treated in a compassionate use program.
Amendment 06	13-Jun-2011	There were errors and omissions in the assessments for the Long-Term Follow-Up in protocol amendment 5, Section 12.4.3, which were corrected and detailed within this amendment.
Amendment 07	05-Jun-2012	Clarification of SAE reporting procedure; addition of Health Outcomes measurements: PedsQL Scale; addition of protocol and study results provision; addition of trademark information tables and update to table numbers.

PEG=polyethylene glycol; ADA adenosine deaminase; GSK=GlaxoSmithKline; ADA-SCID= severe combined immunodeficiency due to adenosine deaminase deficiency; SAE=serious adverse event; PedsQL=Paediatric Quality of Life Inventory.

The protocol amendments appear to be clear and although there have been a number of revisions to the protocol during 2001 and 2012, the changes are not deemed to significantly impact on the study outcomes.

Baseline data

In the pivotal population, the mean age at the time of gene therapy was 2.38 years and the median age was 1.7 years (range: 0.5 to 6.1 years). The approximate age of subjects at the time of ADA-SCID diagnosis ranged from 1 month to 1.25 years. Most subjects were white and 58% were male. The median subject age at the time of gene therapy for all subjects in the integrated population was 1.7 years (range: 0.5 months to 6.1 years). The approximate age at the time of ADA-SCID diagnosis for

subjects across all studies and the CUP ranged from <1 month to 1.25 years. Most subjects across all studies were White and 61% were male, see table below.

Summary of demographic characteristics at baseline/date of gene therapy

Demographic	Pivotal Population (N=12)	Integrated Population (N=18)
Age at gene therapy, n	12	18
Median years (min-max)	1.70 (0.5-6.1)	1.70 (0.5-6.1)
Sex, n	12	18
Female, n (%)	5 (42)	7 (39)
Male, n (%)	7 (58)	11 (61)
Height, n	8	14
Median cm (min-max)[a]	83.4 (66-110)	81.5 (70-114)
Weight, n	12	17
Median kg (min-max)[a]	10.3 (4-21)	10.2 (6-22)
Race, n (%)	12	18
White – White/Caucasian/European heritage	8 (67)	10 (56)
White – Arabic/North African heritage[b]	3 (25)	5 (28)
African American/African heritage	0	2 (11)
Asian – Central/South Asian heritage[b]	1 (8)	1 (6)

Abbreviations: max = maximum; min = minimum

a. Height and weight recorded at screening visit.
b. Two subjects (Subjects [redacted]) had their race incorrectly identified as White/Caucasian in the 0-3 years data set of AD1115611, which was later corrected to Asian and White/Arabic, respectively, in the integrated data set

The clinical program enrolled an international population, with subjects from the EU, Middle East, Africa, North America and South America.

Numbers analysed

Available data for subjects treated with GSK2696273 are reported for the pivotal population (N=12) and the integrated population (N=18).

Outcomes and estimation

Primary endpoint

Survival: Survival was the primary efficacy endpoint in the pivotal study (AD1115611). A 100% survival rate has been observed for all subjects (N=18) who received GSK2696273 treatment in the pivotal and supportive studies and the CUP, with a median follow-up time of approximately 7 years. This survival rate exceeds the most recent published historical comparative rate of 67% overall survival observed in 15 patients who received a MUD hematopoietic SCT after a median of 6.5 years of follow-up (cited for all SCT recipients regardless of donor source). The majority of deaths reported in the historical comparison by Hassan et al occurred within the first 100 days post-SCT and were due to transplant related complications such as graft versus host disease or severe infections [Hassan, 2012]. Survival also exceeds the 86% and 83% rate for recipients of HLA-matched sibling (N=42) and family (N=12) donor SCT, respectively, in the Hassan report.

Intervention-Free Survival: Intervention-free survival was defined as survival without post-gene therapy PEG-ADA use for a continuous period of ≥3 months, SCT, or death. No deaths have occurred in any study. Intervention-free survival represents a sensitivity analysis of the overall survival rate. Within the Pivotal population, 1 subject required PEG-ADA post-gene therapy. Two additional subjects

from supportive studies (on in pilot study 2, on in CUP) required continuous PEG-ADA post-gene therapy. The summary of intervention-free survival is presented in the table below.

Summary of intervention-free survival (pivotal and integrated populations)

	Pivotal Population[a] (N=12)	Integrated Population[b] (N=18)
n	12	17
Survived without intervention, n (%)	11 (92)	14 (82)
Follow-up ongoing, n (%)	11 (92)	14 (82)
Follow-up ended, n (%)	0	0
Intervention or death, n (%)	1 (8)	3 (18)

a. All available data are included, including any data collected beyond Year 8 of follow-up.
b. For all subjects except Subject [redacted] (originally in Pilot Study 1 [AD1117054]), all available data are included, including any data collected beyond Year 8 of follow-up.

Two subjects each received a post-gene therapy HLA-matched sibling donor SCT (one subject in pivotal study and one subject in CUP), both of whom had started continuous PEG-ADA prior to withdrawing from their respective study to receive a SCT. The 92% and 82% intervention-free survival rates in the pivotal and integrated populations, respectively, compare favourably with the 67% overall survival rate following MUD SCT in the historical reference [Hassan, 2012]. Comprehensive reference data on intervention-free survival following SCT are not available; however, the 67% overall survival percentage includes one subject (7%) who required a second transplant [Hassan, 2012]. In addition, Hassan et al reported 52% overall survival among 52 children who received transplants from donor sources other than matched siblings or family members (i.e., the proposed indicated population for GSK2696273). Nine of these patients went on to receive at least one additional transplant, indicating that less than half of subjects receiving non-sibling/family matched SCT survived the transplant procedure without the need for additional intervention. However, Hassan et al did not report on patients (if any) who required reintroduction of PEG-ADA in addition to repeat transplantation.

The primary endpoint in this study both in the pivotal population and the integrated population appears to show 100% survival as there were no deaths. However, it should be noted that the integrated population included those patients who were considered to have failed gene therapy and therefore required PEG-ADA for > 3 months and/or HSCT. This situation is similar to the earlier gene therapy trials in ADA SCID which were confounded by the concomitant use of PEG-ADA. Accordingly, intervention-free survival would therefore be a more clinically meaningful endpoint, which the applicant has additionally analysed in the summary of clinical efficacy as this has not been done in the individual CSRs. The intervention free survival in the pivotal trial appears to be 92% (11/12). However, the applicant excluded from the analysis patients who required PEG ADA for >3 months. The applicant justified this with the fact that a conservative safety approach was taken when the patient suffered from autoimmune hepatitis and accordingly, the ERT was started until further data were available which showed adequate detoxification when ERT was stopped.

Although not included in the analysis because of the data cut-off date, another patient received PEG-ADA after gene therapy, bringing the total number of patients to 5/20 (25%) who needed to restart PEG-ADA after gene therapy, which appears to be remarkably similar to published figures in the recent literature. Accordingly, the observed figures of 92% (11/12) in the pivotal trial and 83% (14/17) in the integrated population would appear to be optimistic. It is acknowledged that survival was the main primary endpoint and intervention-free survival was carried out as a sensitivity analysis. Nevertheless, at this stage it is not possible to predict which patients will respond to gene therapy and further long term follow up in the registry is considered necessary before firm conclusions can be drawn. It should be noted that initial trials in gene therapy were confounded due to concomitant administration of PEG-

ADA and therefore it is important to ascertain responders to gene therapy. The applicant will evaluate intervention-free survival in the registry, as requested by the CAT/CHMP.

Due to these assumptions, the applicant provided an update on the results of intervention free survival during the evaluation procedure, see table below. A 100% survival rate continues to be observed for all subjects (N=21) who received GSK2696273 treatment. This includes the 18 subjects included in the MAA and 3 new subjects treated in a Named Patient Program (NPP) since the clinical cut-off for the MAA. The data presented in the below table is based on follow up data obtained in February 2016.

Rate of intervention-free survival data (including 95% CI) for all treated subjects (Feb 2016)

Summary of Kaplan-Meier Analysis of Intervention Free Survival

Event Time (Years)	Intervention Free Survival Rate (95%)	Cumulative Number of Deaths/Patients Requiring Intervention	Number of Patients at Risk
0.38	0.94 (0.68, 0.99)	1	18
0.47	0.89 (0.64, 0.97)	2	17
0.82	0.84 (0.59, 0.95)	3	16
4.48	0.78 (0.52, 0.91)	4	13

Note:

1. Subject [redacted] originally treated in Pilot Study 1 is excluded from this table. Data collected within Pilot Study 1 is not available in the clinical database and therefore, intervention could not be determined for this patient.
2. Subject [redacted] (NPP) has data up to 0.27 years post treatment and thus is not considered at risk at 0.38 years when the first subject to have an intervention (Subject [redacted] started PED-ADA. Therefore, Subject [redacted] has been excluded from the analyses.

Based on these latest data, 22 patients have been treated with Strimvelis although the duration of the follow-up period for the most recently treated patients have been too short to be included in the overall assessment. Moreover, the one subject was also excluded since its data come from the pilot study 1 for which the available information is limited. This is supported. As a consequence, the calculated variable with the data from 19 patients resulted in a rate of intervention-free survival (95% CI) of 0.78 (0.52, 0.91). This result is quite similar to that provided at an earlier stage of the evaluation procedure for the integrated population, which was 0.81 (0.53, 0.94). However, further data will be obtained from the post-approval registry committed to by the applicant. This is considered acceptable to the CAT/CHMP.

Key secondary endpoints

Severe infections: Change in the rate of severe infections (those that led to hospitalization or prolonged hospitalization) at year 3 post-gene therapy was a key secondary efficacy endpoint in the pivotal study AD1115611. Infections recorded during the first three months post-gene therapy are not included in the analysis because the subjects were hospitalised during most of this time; however, if their per protocol hospitalisation period was prolonged due to infection, this was captured in the severe infection analysis. Within the pivotal population, the rates of severe infections were reduced post-gene therapy when compared with the pre-gene therapy period, see table below. The pre-gene therapy infections may have been under-reported as they were collected as part of the patient history and screening (including caregiver-recalled infections from birth up to the time of gene therapy) rather

than prospectively reported; however, it is notable that the post-gene therapy severe infection rates declined each year following gene therapy.

Summary of Severe Infections Pre- and Post-Gene Therapy (Pivotal and Integrated Populations)

	Pivotal Population (N=12)		Integrated Population[a] (N=18)	
	Pre-GT	Post-GT[b]	Pre-GT	Post-GT[b]
n[a]	12	12	17	17
Number of patients with events, n (%)	10 (83)	8 (67)	14 (82)	10 (59)
Number of events				
Total	29	12	40	15
4 months to 3 years follow-up[b]		9		12
4 to 8 years follow-up		3		3
Person-years of observation (free from infection)				
Total	26.37	71.63	34.30	89.23
4 months to 3 years follow-up[b]		34.20		45.81
4 to 8 years follow-up		37.43		43.42
Rate of infection[c]				
Total	1.10	0.17	1.17	0.17
4 months to 3 years follow-up[b]		0.26		0.26
4 to 8 years follow-up		0.08		0.07
Number of occurrences per patient, n (%)				
n	10	8	14	10
1	3 (30)	6 (75)	4 (29)	7 (70)
2	2 (20)	0	4 (29)	1 (10)
≥3	5 (50)	2 (25)	6 (43)	2 (20)

Abbreviations: GT = gene therapy.
Note: Only data collected prior to PEG-ADA intervention (≥3 months of treatment with PEG-ADA) are included.
a. Subject [redacted] (Pilot 1 Study) is excluded from this analysis as this subject's data regarding severe infections prior to Year 13 are not included in the clinical database.
b. Excludes 3-month hospitalization period post-gene therapy.
c. Rate of infection estimated as number of infections over person-years of observation (free from infection).

The majority of severe infections were reported during the 3-year follow-up period, and the rate declined in LTFU from Years 4 to 8. Following gene therapy, the majority of subjects had only one occurrence of severe infection, which was a marked reduction from the pre-treatment period in which half of the subjects who experienced severe infections had multiple occurrences.

A total of 15 severe infections were reported after GSK2696273 treatment and most of these infections (12/15 events) occurred during the 3-year follow-up, which is not unexpected as immune reconstitution occurs over time and because subjects' central venous catheter (CVCs), which can become infected, remained in place long-term during the 0-3 years follow-up period. All severe infections were reported as resolved. The most frequently reported severe infections were device related infections (n=5) and gastroenteritis (n=3); the device related infections were expected due to long-term placement of CVCs, and gastroenteritis is a common childhood illness. Of note, 2 subjects reported Varicella infection and 1 subject had Staphylococcal sepsis. Severe infections were reported as SAEs and are discussed in section 3.4.

There are obvious differences in the pre and post gene therapy figures for severe infections with respect to the number of events, person years of observation free from infection, rate of infection and number of occurrences per patient. Although the pre-treatment data were retrospectively collected, the decrease observed over time can be considered clinically relevant. It is also notable that most of the

infections appear to be in the first 3 years but decrease from year 4 to year 8 follow up. The majority of infections were CVC device related, although infections included gastroenteritis, varicella and staphylococcus sepsis. The figures appear to be consistent with gradual immune reconstitution following gene therapy.

Immune Reconstitution: Evidence of immune reconstitution after gene therapy was evaluated by changes over time in peripheral lymphocyte counts (T cells, B cells, and NK cells) thymic activity (TREC), peripheral T cell function (proliferation in response to proliferative stimuli) diversity in V-beta repertoire), and B cell function (assessed indirectly by immunoglobulin production, vaccination responses and IVIG use during follow up. The key immune cell types evaluated were:

- CD3+ T lymphocytes: also referred to as T cells
- CD3+ CD4+ T cells: includes
- CD4+ T helper cells: initiate immune responses
- CD4+ T regulatory cells: regulate immune responses
- CD4+ CD45RA+ T cells: naïve T helper cells
- CD3+ CD8+ T cells: cytotoxic T cells
- CD16+ CD56+ cells: NK
- CD19+ B lymphocytes: also referred to as B cells

Lymphocyte counts: Change from baseline in T cell counts was a key secondary efficacy endpoint in the pivotal study (AD1115611). Within the pivotal population, lymphocytes in general and CD3+ T cell counts in particular were increased compared to baseline, see table below. This clinically relevant increase was demonstrated from Year 1 post-treatment and maintained throughout the duration of follow-up. In contrast, changes from baseline were variable for CD19+ B cells and CD16+ CD56+ NK cells, with counts for both cell types decreasing from baseline to Year 1 and then increasing above the Year 1 counts from Year 2 onwards.

MMRM Analysis of Change from Baseline in Log-Transformed Lymphocytes

Cell marker Visit	Pivotal Population (N=12)				Integrated Population (N=18)			
	n	Adjusted mean of ratio	95% CI	P-value	n	Adjusted mean of ratio	95% CI	P-value
CD3+								
Year 1	10	3.18	(2.16, 4.67)	<.001	13	3.84	(2.67, 5.52)	<.001
Year 2	10	4.83	(3.29, 7.10)	<.001	13	5.89	(4.10, 8.48)	<.001
Year 3	10	5.95	(4.05, 8.74)	<.001	12	7.24	(5.01, 10.48)	<.001
Year 4	7	4.74	(3.13, 7.17)	<.001	8	5.92	(3.98, 8.80)	<.001
Year 5	9	5.74	(3.88, 8.50)	<.001	10	6.69	(4.57, 9.79)	<.001
Year 6	5	7.95	(5.06, 12.48)	<.001	6	10.50	(6.88, 16.02)	<.001
Year 7	5	6.16	(3.92, 9.68)	<.001	5	7.49	(4.81, 11.67)	<.001
Year 8	2	4.72			3	6.22	(3.71, 10.43)	<.001
CD19+								
Year 1	10	0.28	(0.14, 0.56)	<.001	13	0.52	(0.28, 0.97)	.040
Year 2	10	0.47	(0.24, 0.95)	.037	13	0.88	(0.47, 1.65)	.690
Year 3	10	0.58	(0.29, 1.16)	.120	12	1.08	(0.57, 2.06)	.804
Year 4	7	0.52	(0.23, 1.17)	.112	8	1.00	(0.47, 2.13)	.989
Year 5	9	0.32	(0.15, 0.66)	.003	10	0.61	(0.31, 1.22)	.162
Year 6	5	0.58	(0.22, 1.49)	.249	6	1.08	(0.46, 2.50)	.864
Year 7	5	0.38	(0.15, 0.99)	.048	5	0.67	(0.27, 1.67)	.386
Year 8	2	0.49			3	0.89	(0.29, 2.79)	.843
CD16+CD56+								
Year 1	10	0.57	(0.23, 1.39)	.205	13	0.60	(0.29, 1.27)	.176
Year 2	10	1.09	(0.45, 2.67)	.837	13	1.08	(0.52, 2.27)	.827
Year 3	10	0.94	(0.39, 2.29)	.890	12	1.12	(0.52, 2.41)	.761
Year 4	7	0.65	(0.24, 1.80)	.399	8	0.78	(0.32, 1.90)	.575
Year 5	9	0.63	(0.25, 1.58)	.311	10	0.69	(0.31, 1.57)	.370
Year 6	5	1.68	(0.53, 5.40)	.372	6	1.73	(0.64, 4.69)	.277
Year 7	5	1.72	(0.54, 5.52)	.354	5	1.79	(0.61, 5.27)	.284
Year 8	2	0.80			3	0.79	(0.20, 3.02)	.721

Abbreviations: CI = confidence interval; MMRM = mixed model repeated measures.
Note: Model includes fixed effects for Visit and Baseline. Patient is fitted as a random effect. The compound-symmetry variance covariance structure was applied. An imputation was applied to any value where the observed value=0 in order to log transform the data. For all biomarkers the observed value was imputed to 1 if the observed value=0. 95% CIs and p-values are not provided if n<3.

T cell subsets: Among the T cell subsets evaluated for the pivotal population, there were sustained increases from baseline in CD4+ (helper and regulatory) T cells and CD8+ (cytotoxic) T cells after gene therapy, particularly from year 1 onwards. Results for CD4+ CD45RA+ naïve T cells (mature T cells capable of responding to newly encountered pathogens) were similar. Similar findings were observed for cell subset analysis in the integrated population. Patients with ADA-SCID have pronounced lymphopenia before treatment. After gene therapy there is generally a steady increase with respect to CD3+T cell reconstitution which is critical for patient survival but this seems to be variable in the case of CD19+ B cells and CD16+ and CD56+ NK cell. Nevertheless, B cell deficiency can be managed with immunoglobulin replacement. The T cell subsets also show consistent changes. However, immune reconstitution after gene therapy appears to be a relatively slow process when compared to SCT.

Immune cell function

T Cell function: T cell development and function were demonstrated by means of thymic activity (T cell receptor recombination during thymic selection assessed by TREC), V-beta T cell receptor chain usage as evidence of diversity in the T cell receptor repertoire, and peripheral T cell proliferation in response to polyclonal stimuli.

Thymic Activity: T cell receptor excision circles (TREC) are DNA fragments formed in T cells during the T cell receptor generation which occurs during the development of T cells in the thymus. They are non-replicative; thus, when immune cells divide in response to antigen the TREC do not. For this reason, their presence in peripheral blood T cells is a useful marker of thymic activity (i.e., production of newly formed naïve CD45RA+ T cells). The contribution of the thymus to immune development in adults has historically been unclear; however, an age related decrease in thymus size and activity is expected as children approach adolescence and the thymus atrophies.

T Cell Receptor Repertoire: In the pivotal population, all subjects had evidence of polyclonal V-beta chains at one or more time points following gene therapy. There were 8 subjects with evidence of a fully normal V-beta repertoire (all individual V-beta chains within the normal range) which remained normal during the LTFU and is indicative of a mature T cell compartment.

Peripheral T cell proliferation: From year 1 onwards the mean and median results in the pivotal population were greater than baseline and demonstrated robust proliferation in response to stimulation (where at minimum, >20,000 counts per minute (cpm) represents evidence of normal proliferation above assay background, typically observed with anti-CD3 stimulation, and values at or exceeding 100,000 cpm are often observed in response to PHA). All available data from LTFU (Years 5 and 8) are shown and indicate sustained robust T cell proliferation; the lower number of subjects with available data may have contributed to the wider confidence intervals at these time points. Similar results were observed in the integrated population, including increases in proliferation in response to both anti-CD3 and PHA polyclonal stimuli.

The TRECs after gene therapy show expected changes with an increase until year 3 followed by gradual decline. The T cell receptor repertoire and T cell proliferation are also supportive of immune reconstitution. It should be noted that TRECs are significantly higher in children aged 2 months to 3 years compared with those 3 to 16 years. The thymus in patients with infantile SCID is vestigial but able to support T-cell maturation from donor stem cells. Accordingly, thymic function would appear to be an important age-related difference for ADA-SCID patients who may present after infancy. Furthermore, ADA-deficient patients may have an accelerated decline in thymic function due to the systemic toxicity. Furthermore, the main difference across paediatric age groups potentially related to the efficacy of the gene product is the decrease in thymic function in older children and adolescents, which could possibly lead to reduced efficacy in them. This has implications for the indication which is too broad as currently proposed and has been addressed by appropriate warnings in section 4.4 of the SmPC, in patients older than 6 years the gene therapy should be reserved for occasions where all other treatment options have been exhausted.

B cell function: Despite B cell counts remaining low after gene therapy (i.e., post-gene therapy B cell counts were not consistently increased, and at some time points decreased, from baseline), functional immune protection was demonstrated by assessment of immunoglobulin chain diversity, the need for IVIG supportive treatment after receiving gene therapy, and vaccine responses.

Immunoglobulins

In the pivotal population, baseline IgA levels were low with a mean value of 0.205 g/L. By Year 1, the mean IgA level had increased to 0.299 g/L and further increases to mean values above 1.1 g/L were observed at Years 6 to 8, which suggest the development of B cells since IgA is not replaced by IVIG. This is supported by increases observed in the post-gene therapy mean values of IgM and IgE over time. Interpretation of the IgG levels is complicated because subjects were receiving IVIG replacement during the initial post-treatment period, as defined by the protocol, and several subjects also received additional IVIG infusions for treatment of AEs. Overall, the mean IgG values were stable over time

from baseline throughout Year 8, over which time many subjects were discontinuing IVIG infusions. Serum immunoglobulin levels of IgG, IgA, IgE, and IgM remained generally stable during LTFU or showed small increases from LTFU baseline at Year 3 to Year 4 and onwards demonstrating long-term function of B cells in antibody production of all subtypes. Serum immunoglobulin levels in the integrated population were supportive of the pivotal population findings.

Requirement for IVIG therapy

In the pivotal population, all 12 subjects were receiving IVIG replacement at the time of screening, and all received post-gene therapy maintenance IVIG. Seven subjects were able to discontinue IVIG replacement during the 0-3 years follow-up. Nine subjects (75%) had discontinued IVIG replacement therapy at the time the data cut-off date (8 May 2014). The median time to IVIG discontinuation after gene therapy was 3 years 4 months (range: 1 year 3 months to 4 years 10 months). Thus, replacement IVIG use declined as follow-up progressed in years 4 to 8 after gene therapy, providing evidence for functional B cell and immunoglobulin production in the periphery. When assessed across all studies, 12 out of 18 subjects had discontinued IVIG treatment at the time of the data cut-off for the MAA and among these subjects, the median time to IVIG discontinuation was 2 years and 2 months (range 7 months to 4 years 10 months).

Vaccination Responses

In the pivotal population, majority of subjects had antibodies to a range of infectious antigens at one or more time points after IVIG had been stopped, which is reflective of B cell antibody forming capacity after gene therapy. In some of the 9 subjects who discontinued IVIG therapy post-gene therapy, detectable antibodies to pertussis, tetanus toxoid, diphtheria, Haemophilus B and hepatitis B surface antigen were detected. Antibodies were generally detectable at multiple time points during follow-up, and in a number of subjects were continuing from the 0-3 years follow-up, suggesting long-lived antibody production.

Physical growth

Development of a functional immune system and a decrease in severe infection rates are critical to ongoing physical growth. Several subjects had height or weight that transiently fell below the curve at individual time points, but the majority either maintained or improved their age-appropriate height and weight relative to standard curves. There were exceptions: one subject had severe baseline disease and autoimmunity considered as contributing factors to failure to thrive, and another subject was below the curve for weight from year 6 through year 8 only. Shifts in growth from above the 5th percentile to below this threshold were uncommon.

Requirement for Post-Gene Therapy PEG-ADA Treatment

A total of 4 subjects (2 in the pivotal population, and 2 in supportive studies) who received GSK2696273 required post-baseline treatment with PEG-ADA. Short-term PEG-ADA was used by 1 subject in the pivotal population who restarted PEG-ADA approximately 2.1 years after gene therapy following an SAE of autoimmune hepatitis. Two further doses of PEG-ADA were administered within the same month along with prednisone. This subject recovered and has not needed PEG-ADA after this episode.

Long-term PEG-ADA (exceeding 3 months of continuous duration) was used by 3 subjects (one in each, the pivotal population, supportive study, and the CUP, all of whom were considered as having had unsuccessful response to gene therapy).

One subject received two doses of gene therapy that did not follow the recommended dosing and preconditioning regimen, which may have contributed to lack of engraftment; the subject remained lymphopenic and required introduction of short-term PEG-ADA starting 2.4 years after her first gene therapy and before a second dose of gene therapy administered 2.6 years after the first gene therapy. Long-term PEG-ADA use began approximately 4.5 years after the first gene therapy and has continued in LTFU.

Another subject had pre-existing chronic haemolytic anaemia requiring corticosteroid treatment prior to and during gene therapy, and restarted PEG-ADA approximately 5 months after gene therapy following an SAE of autoimmune thrombocytopenia. This subject had poor engraftment and remained on PEG-ADA continuously for the duration of follow-up until withdrawal from the study. Additional supportive therapy included granulocyte colony stimulating factor (G-CSF; lenograstim/filgrastim) for neutropenia, methylprednisolone, and prednisone. This subject became a candidate for sibling donor allogeneic SCT and was moved to another clinical centre after study withdrawal.

The third subject 17 restarted PEG-ADA 0.34 years after gene therapy. This subject received further PEG-ADA intermittently on additional occasions due to poor immune reconstitution, then continuously through the end of the 0-3 year follow-up period. This subject became a candidate for sibling donor allogeneic SCT after consenting to LTFU participation but prior to the Year 4 visit, and was moved to another clinical centre after withdrawal from the study.

The summary of patients requiring PEG-ADA post gene therapy is summarised in the table below.

Number and Percent of Subjects Receiving Post-Baseline PEG-ADA (pivotal and integrated populations)

	Pivotal Population (N=12)	Integrated Population (N=18)
n	12	17[a]
No PEG-ADA, n (%)	10 (83)	13 (76)
PEG-ADA <3 months continuous period, n (%)	1 (8)	1 (6)
PEG-ADA continuously ≥3 months, n (%)	1 (8)	3 (18)

Abbreviations: PEG-ADA = polyethylene glycol adenosine deaminase.
a. Data are not available for Subject 1.

Subjects with Unsuccessful Response to Gene Therapy

Lack of response to GSK2696273 treatment is considered to be secondary to poor engraftment of gene modified bone marrow cells and the diminished downstream clinical benefit, including immune reconstitution and correction of the systemic metabolic defect. Subjects who received GSK2696273 and then required post-gene therapy PEG-ADA for >3 months continuously or SCT were excluded from efficacy analyses upon meeting either criterion and are also considered clinically to have experienced unsuccessful response to gene therapy; 3 subjects met these criteria and are summarized in the table below.

Subjects with Unsuccessful Response to GSK2696273 Treatment

Subject/ Study	Treatment Intervention Details	Subject Status
[redacted] AD1117056	Required PEG-ADA for 7 weeks due to lymphopenia 2.4 years after 1st dose of gene therapy, received 2nd gene therapy 2.6 years after 1st dose, long-term PEG-ADA administration started 4.5 years after 1st gene therapy	In LTFU, continues to receive PEG-ADA
[redacted] AD1115611	Subject had prior history of autoimmune AEs and SAE of neutropenia. Required PEG-ADA reintroduction approximately 5 months after gene therapy due to SAE of autoimmune thrombocytopenia following poor response to steroids, IVIG, and platelet transfusions	Withdrew from pivotal study, received subsequent sibling donor SCT with good donor engraftment [Aiuti, 2014, personal communication]
[redacted] AD1117064	Received purified CD34-negative cell fraction due to CMV reactivation and lymphopenia, PEG-ADA reintroduction at 0.34 years after gene therapy	Withdrew from LTFU before Year 4 visit, received subsequent sibling donor SCT with good donor engraftment [Aiuti, 2014, personal communication]

Abbreviations: AE = adverse event; CMV = cytomegalovirus; IVIG = intravenous immunoglobulin; LTFU = long-term follow-up; PEG-ADA = polyethylene glycol modified bovine adenosine deaminase; SAE = serious adverse event; SCT = stem cell transplant.

A summary of potential reasons for unsuccessful response are shown below:

- Unsuccessful response to gene therapy possibly related to the low cellularity at the time of bone marrow harvest and subsequent low gene therapy dose;
- Unsuccessful response to gene therapy possibly related to chronic autoimmunity prior to and after gene therapy that required long-term immunosuppressive treatment.

The reason for the unsuccessful outcome in the third subject is not immediately clear, however, chronic CMV infection prior to gene therapy and reactivation after gene therapy may have contributed to the unsuccessful response to gene therapy. Second and third subjects had received prior PEG-ADA therapy before gene therapy.

However, as ADA is expressed intracellularly, immunogenicity due to pre-existing anti-ADA antibodies that might have arisen from pre-gene therapy treatment with PEG-ADA is considered unlikely to have contributed to the unsuccessful gene therapy in these subjects. Anti-PEG-ADA antibodies were not measured in the GSK2696273 programme. Nevertheless, the effect of inhibitory antibodies with respect to treatment failure cannot be entirely excluded in some cases and accordingly, the applicant agreed to monitor immunogenicity in the patients who will entered in the registry, as requested by the CAT/CHMP. In this respect, antibodies to PEG-ADA will be evaluated initially. However, in those patients in whom inhibitory antibodies are detected, immunogenicity with respect to bovine ADA and human ADA should, additionally, be evaluated. The applicant also commits to develop an appropriate screening, confirmatory and functional assays which have been fully validated for this purpose.

Ancillary analyses

Several additional analyses were conducted on data from the pivotal population for B cells and lymphocyte lineage cells. On B cells, expression of the two types of immunoglobulin light chains (kappa and lambda) was evaluated. The ratio of kappa chain expressing B cells versus lambda chain is normally 0.5 to 3.0. Over the course of post-gene therapy follow-up, the mean percentage of CD19+ B cells expressing kappa chains ranged from 43% to 64%, and for lambda chains ranged from 19% to 37%. These values are in line with the expected ratio CD2 expression on T cells, NK cells. A subpopulation of B cells was evaluated for subjects in the Pivotal population; these cells were present

at baseline due to prior treatment with PEG-ADA. CD2 expressing cells were initially depleted due to preconditioning, increased back to baseline levels between 1.5 to 2 years post-gene restoration of CD2 expressing immune cell populations after treatment with gene therapy. An exploratory responder analysis was conducted to determine the proportion of subjects at each time point who were T cell responders (CD3+ CD4+ count >300x106 cells/L and/or CD3+ >1000x106 cells/L) or B cell responders (CD19+ count >100x106 cells/L) based on cell counts in whole venous peripheral blood. Selection of the cut-off values to define responders was based on criteria reported by Hassan et al in patients receiving conventional SCT [Hassan, 2012]. It is difficult to directly compare post-gene therapy T cell responder data to the values reported by Hassan et al, as the latter only reported immune reconstitution for patients surviving SCT and due to differences in the handling of data for subjects who had post-treatment interventions. Interpretation of results for these responder analyses is therefore limited, but they are in general supportive of the cell count change from baseline analyses, which showed emergence of lymphocytes and significant increases in T cells (including CD4+, CD8+, and naïve subsets) starting from year 1 and maintained thereafter. In the pivotal population, at the 6-month visit there were 0/11 responders, by year 1 between approximately one-quarter and one-third of subjects were B cell and T cell responders, and by year 3, 64% of subjects were T cell responders and 45% of subjects were B cell responders. From years 4 to 8, proportions of subjects who were B cell responders varied (range: 20% to 100%), and the majority of subjects were CD3+ CD4+ T cell responders from year 4 onwards (range: 50% to 100%). In the integrated population, at the 6-month visit there were 0/16 subjects with available data who were responders, and by year 3 (n=14), approximately half of subjects were B cell and T cell responders. By year 5 (n=10), B cell response was 40%, a level that was generally maintained through year 8. The proportion of CD3+ CD4+ T cell responders in year 5 was 80%, and this response level was maintained through year 8. The trend was generally reflected in the CD3+ T cell responder analyses from year 5 onwards. By comparison, Hassan et al reported immune reconstitution data for 55 of 71 surviving transplant patients (all donor types). There were 30% of surviving MUD transplant recipients with data available, who demonstrated T cell recovery to cell counts >1000x106/L (i.e., T cell responder) by 6 months post-transplant, with 71% considered T cell responders by 3 years post-transplant. These kinetics reflects the time required for transplanted T cell precursors to undergo thymic selection and emerge into the periphery as mature T cells. In contrast, 60% of surviving recipients of matched sibling donor transplants who had cell count data available showed this level of T cell recovery at 6 months, and 79% did at 2 years [Hassan, 2012], which can be expected due to receipt of closely HLA-matched mature functioning T cells at the time of transplant. In the GSK2696273 programme, T cell recovery to this level was slower, occurring at least 3 or more years post-gene therapy; however this should be considered in the context of 100% overall survival in this population. B cell recovery was assessed by Hassan et al primarily by cell function rather than responder analysis.

Overall, the exploratory responder analysis with respect to immune reconstitution appears to show a slower immune recovery after gene therapy compared to SCT (1 year versus 6 months respectively) and for levels of response around 80%, the corresponding figures are 3 years versus 2 years. This, however, is to be balanced out by the level of transduction and superior survival observed with gene therapy.

Summary of main study

The following tables summarise the efficacy results from the main study supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Summary of efficacy for trial AD1115611

Title: ADA gene transfer into hematopoietic stem/progenitor cells for the treatment of ADA-SCID			
Study identifier	AD1115611		
Design	Open-label, prospective, sequential study in children with SCID due to ADA deficiency who lacked a healthy HLA-identical sibling.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		3 years not applicable All subjects completing this study were offered the opportunity to be followed up for a further 5 years
Hypothesis	Superiority (over historical control group)		
Treatments group			12 subjects were enrolled to the test treatment.
Endpoints and definitions	Primary endpoint	3-year Survival	
	Secondary endpoint	Change in rate of severe infection	Rate of infection estimated as number of infections over person-years of observation (free from infection) after the first 3 months after treatment administration (after gene therapy) or before gene therapy.
	Secondary endpoint	Change in T-lymphocyte count	Comparison of CD3+ T lymphocytes between baseline and Year 3
	Secondary endpoint	Metabolic detoxify-cation	Adequate 'systemic' metabolic detoxification was classified as levels of purine metabolites (dAXP=dAMP+dADP+dATP) in RBC <100 nmol/mL.
Database lock	8 November 2012 (database unfrozen on 13 November 2012 to make corrections to the laboratory, normal range and biomarker and re-frozen on 28 November 2012. Additional changes or corrections to the database were identified during the review of data displays which required unfreezing and refreezing of the database. The final refreeze of the database for this study report was performed on 16 August 2013. These changes included, but were not limited to, prior medical history records to capture pre-treatment severe infections, and the reason for withdrawal for 1 subject.		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	Treatment group	Strimvelis Baseline	Strimvelis Year 3	
	Number of subject	12	12	

	Overall Survival at 3 years (<statistic>)	N/A	100%	
	Severe infection rate	1.1	0.429	0.005
	CD3+ T lymphocytes GM ratio	3.0	6.5(0.38)	CI 3.08-13.64 <0.001
	Metabolic detoxification Y or N	0	11/11(100%0	CI 72-100% <0.001

Analysis performed across trials (pooled analyses and meta-analysis)

Considering the limited number of patients treated with Strimvelis, the combined analyses for both the pivotal and integrated population are integrated into the individual sections of this report.

Clinical studies in special populations

This section is not applicable for the population treated with Strimvelis.

Supportive studies

Long-Term Follow-Up (AD1115611 LTFU): Efficacy assessments in the LTFU component of AD1115611 included survival, clinical and biochemical evaluations, and evidence of engraftment similar to the assessments in the pivotal study. Analysis methods for efficacy endpoints were analogous to those utilized in the pivotal study analysis up to 3 years of follow-up, but included comparisons to the 3-year time point (considered as the LTFU baseline). Since subjects enrolled in the contributing studies at different times, the LTFU study is ongoing as of the timing of the MAA submission with varying length of follow-up for subjects. All available efficacy data collected up to and including the year 8 visit at the time of the cut-off date (08 May 2014) were included in the integrated summaries of efficacy data and are discussed in the individual sections.

Pilot Study 2 (AD1117056): The study captured data for 2 subjects treated with GSK2696273 in an open-label, single-arm study. Details of study administration were not defined a priori. The study population included patients with ADA-SCID who lacked an HLA-identical sibling, had received ≥6 months PEG-ADA treatment (except in cases of PEG-ADA allergy), and had demonstrated failure to PEG-ADA therapy. However, neither of the 2 subjects received PEG-ADA before gene therapy because it was not available in their country. Post-treatment efficacy assessments consisted of follow-up for survival, clinical and biochemical evaluations, and evidence of engraftment. These were defined as endpoints post hoc and are similar to the assessments in the pivotal study.

Pilot Study 1 (AD1117054): GSK2696273 was first administered to humans in 2000 in a pilot clinical study with one subject with confirmed ADA-SCID who had no HLA-identical sibling or prior ERT therapy received treatment and follow-up at Hadassah University (Jerusalem, Israel), with the cooperation of HSRTIGET. The report encompasses 13 years of follow-up after GSK2696273 treatment for this subject. Due to data documentation issues this subject contributes only the date of gene therapy for

the integrated efficacy analysis. From year 13 onward, at which time the subject was consented to AD1115611 LTFU, additional LTFU data are included for the integrated efficacy analysis.

Compassionate use program CUP (AD1117064): Compassionate use of gene therapy for ADA-SCID was initiated in 2010. Three subjects received GSK2696273, all of whom had baseline medical conditions comparable to the enrolment criteria for the pivotal study. Assessments and procedures described in the protocol for AD1115611 were followed as stipulated by the approving Ethics Committee. Although the programme was not designed with prospectively defined safety and efficacy endpoints, GSK retrospectively sought and received ethics approval and patient/caregiver consent to use these data for registration.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The ADA-SCID is an ultra-rare disease with an incidence ranging from 0.22 to 0.68 per 100,000 live births or less than 50 paediatric cases in the US and EU combined, which severely limits the possibility of large scale randomised clinical trials. All clinical studies in the GSK2696273 programme were non-randomised, single-arm, open label studies. Therefore, the comparison of interest for efficacy endpoints was considered as within-subject, between pre- and post-treatment assessments; the exception was the endpoint of survival, which was compared to an historical reference [Hassan, 2012]. In view of the rarity of the disease this was considered acceptable to the CAT/CHMP.

The patient population included in the study is appropriate; the age range of patients varied from 0.5 to 6.1 years. The primary and secondary endpoints are acceptable with respect to this patient population as they incorporated patients who were intolerant to PEG-ADA, refractory to PEG-ADA or naïve to PEG-ADA. In the clinical trials, 3 subjects who were not previously treated with PEG-ADA received GSK2696273, and 2 additional subjects received GSK2696273 after 2 months or less of being treated with PEG-ADA. Four out of these five subjects had a successful response to gene therapy. Failure of PEG-ADA treatment was defined by immune parameter alterations such as absolute lymphopenia (<1500 cells/μL), absolute T lymphopenia (<1000 cells/μL), and deficits of serum immunoglobulins. In the GSK2696273 clinical development programme, 3 subjects had not been treated with PEG-ADA before receiving gene therapy; the remaining 15 subjects had prior PEG-ADA exposure, ranging from a few weeks to over 5 years. Per protocol, all the latter subjects discontinued ERT before treatment, to allow evaluation of the gene therapy alone. The applicant clarified that baseline samples were taken when the subjects were still receiving PEG-ADA and indicated a good correction of the metabolic defect as measured by erythrocyte dAXP levels. For this reason, PEG-ADA was used as rescue therapy in some cases.

A single centre participated in the study what assures internal validity but the applicability outside the Hospital San Raffaele Telethon Institute for Gene Therapy is compromised. The current proposed product shelf-life (6 hours) is too short to support shipping to other European clinical centres. The study sought to recruit subjects who had largely exhausted other treatment options. The duration of the pivotal study over 3 years followed by a long term follow up phase is considered adequate. The baseline assessments for each subject were used to evaluate change from baseline for all efficacy endpoints for the integrated analyses. Four subjects had received a prior unsuccessful SCT from a haplo-identical donor, 2 from the pilot study 2, and 2 from the pivotal study. Fifteen subjects had received pre-baseline PEG-ADA therapy; among these, 10 subjects received PEG-ADA at the time of the screening visit. PEG-ADA was discontinued approximately 2-3 weeks before gene therapy.

Given the very long history of the development programme which was started in 1990, it is recognised that some elements are not consistent with current standards of GCP. In particular, substantial supporting study documents for the 2 pilot clinical studies are not available. Furthermore, three of the subjects reported in this MAA received treatment under a named patient "compassionate use" programme and were not initially part of a clinical trial. The pivotal study protocol, amendments, informed consent, and other information that required pre-approval were reviewed and approved by a national, regional, or investigational centre ethics committee or institutional review board, in accordance with GCP and applicable country-specific requirements. Although there have been a number of revisions to the protocol during 2001 and 2012, the changes are not deemed to significantly impact on the study outcomes. A routine GCP inspection of the pivotal trial was also requested by the CAT/CHMP. The findings noted during the inspection showed that GCP was not fully implemented as a quality standard during the academic phase of the study and the documentation of the informed consent process regarding patients from other countries was limited. However, the findings do not question the reliability and integrity of the overall data generated during the trial. This is therefore acceptable to the CAT/CHMP.

Efficacy data and additional analyses

The primary endpoint in this study both in the pivotal population and the integrated population appears to show 100% survival as there were no deaths. However, it should be noted that the integrated population included those patients who were considered to have failed gene therapy and therefore required PEG-ADA for > 3 months and/or HSCT. This situation is similar to the earlier gene therapy trials in ADA SCID, which were confounded by the concomitant use of PEG-ADA. Accordingly, intervention-free survival is therefore a more clinically meaningful endpoint, which the applicant has additionally analysed. It is also worth noting that for the purposes of comparison, the 20-year survival data for patients receiving PEG ADA was 78%, which is very similar to that of the two mentioned populations. However, the follow-up period for the gene therapy was much shorter. The survival rate exceeds the most recently published historical comparative data of 67% in overall survival that was observed in 15 patients who received MUD hematopoietic SCT after a median of 6.5 years of follow-up (Hassan et al 2012). Intervention free survival in the pivotal trial appears to be 92% (11/12). However, the applicant has only excluded patients who required PEG-ADA for >3 months from the analysis and it was acknowledged that some other patients received PEG-ADA albeit for less than 3 months. Hence, the intervention-free survival will be kept under review in the registry, as requested by the CAT/CHMP, and the applicant has committed to do this.

With respect to the key secondary endpoint of severe infections, there are obvious differences in the pre- and post-gene therapy figures for severe infections with respect to the number of events, person years of observation free from infection, rate of infection and number of occurrences per patient. It is also notable that most of the infections appeared in the first 3 years, and they decreased from year 4 to year 8 of the follow up. The majority of infections were CVC device related, although infections included gastroenteritis, varicella and staphylococcus sepsis. The figures appear to be consistent with gradual immune reconstitution following gene therapy.

Patients with ADA-SCID have pronounced lymphopenia before treatment. After gene therapy, there is generally a steady increase with respect to CD3+T cell reconstitution which is critical for patient survival but this seems to be variable in the case of CD19+ B cells and CD16+ and CD56+ NK cell, nevertheless, B cell deficiency can be managed with immunoglobulin replacement. The change from baseline in the log-transformed T lymphocytes (CD3+x106/L) showed an adjusted mean ratio of 3.18, 4.83, 5.95 and 4.74 at years 1, 2, 3 and 4 of follow-up, respectively. In contrast, the change from baseline in log-transformed T lymphocytes (CD16+CD56+x106/L) showed an adjusted mean of ratio

0.57, 1.09, 0.94, and 0.65 in the first four years, and to CD19+ x106/L of 0.28, 0.47, 0.58 and 0.52 at time point of 1, 2, 3 and four years, respectively, suggesting lack of response. The T cell subsets also showed consistent changes. However, immune reconstitution is a relatively slow process and is dependent on the cell type, although it seems to be slower than that observed after SCT.

With respect to B cell function, the increase in serum IgA would appear to indicate endogenous synthesis since IgA is not present in replacement immunoglobulin, which however, complicates evaluation of the synthesis of IgG. These changes appear to persist long term, since a substantial proportion of the patients discontinued IVIG and showed detectable antibodies to a range of infection antigens as well as generating an antibody response to live attenuated vaccination. Modification of the systemic metabolic defect, which was also a key secondary endpoint, is evident from year 2 with 100% response with persistence of effect. Some supportive data is also provided by growth curves as well as quality of life measurements, albeit non-standardised and informal. The applicant acknowledged that there is limited experience about recommending the pre-treatment conditioning with busulfan. In the frame of the agreed long-term follow up post-authorisation registry, the applicant has committed to conduct an exploratory analysis in order to investigate the relationship between busulfan AUC, captured at baseline, and clinical outcome (e.g. survival, intervention free survival and lymphocyte count differentials).

2.5.4. Conclusions on the clinical efficacy

From the totality of data it is abundantly clear that there is compelling evidence for efficacy which has been conclusively demonstrated in clinical practice, since patients with early and delayed onset of ADA-SCID rarely survive beyond 1 to 2 years unless immune function is fully restored either with SCT or PEG-ADA. With respect to the primary endpoint, both, the pivotal trial population as well as the integrated population show superior survival as well as intervention free survival compared to SCT and long term PEG-ADA administration. The key secondary endpoints provide additional data which is consistent with the increased survival.

The CAT considers the following measures necessary to address issues related to efficacy:

- The applicant will provide the final study report of the long term follow up study AD1115611 LTFU as an obligation. In this respect all 18 patients should be followed up for a period of 8 years, Category 3, RMP

Recommendation: In addition, the CAT recommended that the applicant works to develop a state of the art methodology which can be employed to assess retroviral insertion site clonality in patients treated with Strimvelis.

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

2.6. Clinical safety

Patient exposure

A total of 20 subjects have been treated with GSK2696273 gene therapy. However, 2 subjects (were treated with GSK2696273 after the integrated data cut-off (08 May 2014); therefore, exposure and disposition data for these 2 subjects are not included in the data displays and analyses. The range of doses was 0.90 x 106 to 18.15 x 106 CD34+ cells/kg. There were 2 cases of contaminated drug product. These resulted in alterations in the timing of GSK2696273 administration in 2 subjects, as described below, and adjustments to busulfan dosing in these 2 subjects. At the time of the dossier submission, 16 subjects were ongoing in the GSK2696273 clinical development programme. The

median duration of follow-up is approximately 7 years. Two subjects have prematurely withdrawn due to unsuccessful responses to gene therapy.

Summary of subject disposition and duration of follow-up (All studies and CUP safety population)

	Gene therapy (N=18)
Completion Status, n (%)	
Ongoing	16 (89)
Died	0
Prematurely withdrawn	2 (11)
Primary reason for withdrawal, n (%)	
Investigator discretion	1 (6) [a]
Lack of efficacy	1 (6) [a]
Duration of follow-up	
n	18
Median (min, max), years	6.94 (2.3-13.4)

a. The reason for withdrawal of Subject [redacted] was recorded as 'investigator discretion' in the CRF and source tables. Subjects [redacted] and [redacted] had unsuccessful responses to gene therapy

Summary of exposure to GSK2696273 gene therapy (All studies and CUP safety population)

Exposure parameter	Gene therapy (N=17)[a, b]
Total volume infused (mL)	
Mean (SD)	26.18 (7.32)
Median (range)	26.00 (10.0-40.0)
Total nucleated cells (x 10^6)/ kg	
Mean (SD)	21.72 (7.73)
Median (range)	23.10 (2.4-37.0)
Number CD34+ cells/kg (x 10^6/kg)	
Mean (SD)	8.94 (4.04)
Median (range)	9.46 (0.90-18.15)

a. Data from Subject [redacted] are not included in this summary of exposure to GSK2696273.
b. Subject [redacted] received gene therapy on 2 separate occasions, but only data from the first are included in this summary of exposure.

The safety population was essentially the same as efficacy population, except that 2 patients started treatment after the data cut-off. However, only SAEs have been reported. Although it would be logical to consider AEs related to busulphan in the safety assessment, it should be noted that the dose of busulphan was significantly less than that used in a myeloablative regimen and therefore, the AEs expected may not be entirely consistent with the typical safety profile. It is, however, noted that one patient had an unsuccessful response to gene therapy following treatment with and without conditioning treatment with busulphan.

Adverse events

All 18 subjects in the all studies and CUP safety population, as well as all subjects in the various post-treatment study phases reported AEs on or after gene therapy, see table below. AEs were predominantly grade 1 and 2. No deaths or fatal events were reported. Fifteen subjects in the

integrated database had SAEs. None of the SAEs were considered by the investigator to be related to study treatment; 1 subject had 2 AEs that were considered by the investigator to be possibly related to study treatment (hepatic steatosis and white blood cell analysis abnormal).

Overview of AEs (All studies and CUP safety population)

	GSK2696273
Pre-treatment phase	
Any pre-treatment AE, n (%)	17 (94)
Total number of pre-treatment AEs	123
Treatment and post-treatment phases	
Any AE on or after gene therapy, n (%)	18 (100)
Total number of AEs on or after gene therapy	586
Deaths	0
Non-fatal SAE, n (%)	15 (83)
Total number of SAEs on or after gene therapy	39
Treatment-related SAEs	0
Any AE by study phase, n (%)	
Treatment	7 / 17 (41)
3-month hospitalization	17 / 17 (100)
3 months to 3 years follow-up	17 / 17 (100)
4-7 years follow-up	13 / 13 (100)
≥8 years follow-up	6 / 6 (100)
Any AE by severity, n (%)	
Grade 1	18 (100)
Grade 2	17 (94)
Grade 3	15 (83)
Grade 4	5 (28)
Missing severity	1 (6)
Any treatment-related AE (per investigator judgment), n (%)	1 (6)
Any AE resulting in study withdrawal	0

Abbreviations: AE = adverse events; NPP = Named Patient Programme; SAE = serious adverse events
Notes: Subjects are included in the denominator if follow-up is ongoing for the period considered. Subject [redacted] started follow-up at Year 13 but has AEs reported at Year 8 and Year 12, which are included.

In the Medical Dictionary for Regulatory Activities MedDRA System Organ Classes (SOCs), the most frequently reported AEs were infections and infestations, investigations, blood and lymphatic system disorders, and skin and subcutaneous tissue disorders. For each of these SOCs, the AE density (exposure adjusted incidence of events per 100 patient years) was greatest during a phase before (pre-treatment phase) or during hospitalization (treatment and 3- month hospitalization phases) compared with the AE densities recorded for follow-up phases beginning 3 months post GSK2696273 gene therapy and later. AEs of interest, including infections, neurological, immune reactions, oncogenesis, and hepatic are discussed later. In the blood and lymphatic system disorders SOC, anaemia, neutropenia, febrile neutropenia, thrombocytopenia, bone marrow failure, and/or pancytopenia were reported in 11 subjects, see table below.

Summary of AEs reported in 3 or more subjects, by system organ class and preferred term (integrated population)

System Organ Class Preferred Term, (data presented as n [%])	Pre-Treatment (N=17)	Treatment (N=17)	3-Month Hospitali-zation (N=17)	3 Months to 3 Years Follow-up (N=17)	4-7 Years Follow-up (N=13)	≥8 Years Follow-up (N=6)	Total (N=18)
Infections and infestations	12 (71)	2 (12)	14 (82)	17 (100)	12 (92)	5 (83)	18 (100)
Upper respiratory tract infection	1 (6)	0	3 (18)	8 (47)	5 (38)	1 (17)	12 (67)
Gastroenteritis	2 (12)	0	2 (12)	8 (47)	2 (15)	0	10 (56)
Rhinitis	2 (12)	0	0	8 (47)	3 (23)	0	9 (50)
Bronchitis	0	0	1 (6)	5 (29)	3 (23)	0	6 (33)
Device-related infection	0	0	3 (18)	4 (24)	0	0	6 (33)
Ear infection	1 (6)	0	1 (6)	3 (18)	2 (15)	0	6 (33)
Oral candidiasis	3 (18)	0	4 (24)	2 (12)	1 (8)	0	6 (33)
Nasopharyngitis	1 (6)	0	2 (12)	4 (24)	0	0	5 (28)
Pneumonia	0	0	1 (6)	1 (6)	2 (15)	1 (17)	5 (28)
Sinusitis	2 (12)	0	0	2 (12)	5 (38)	0	5 (28)
Urinary tract infection	0	0	2 (12)	3 (18)	1 (8)	2 (33)	5 (28)
Candida infection	2 (12)	0	1 (6)	1 (6)	0	0	4 (22)
Otitis media	0	0	1 (6)	2 (12)	0	1 (17)	4 (22)
Pharyngitis	0	0	0	1 (6)	2 (15)	0	4 (22)
Varicella	0	0	0	3 (18)	1 (8)	0	4 (22)
Escherichia urinary tract infection	0	0	1 (6)	3 (18)	1 (8)	0	3 (17)
Fungal skin infection	0	0	2 (12)	0	0	1 (17)	3 (17)
Haemophilus infection	0	0	0	3 (18)	0	0	3 (17)
Influenza	0	0	0	1 (6)	2 (15)	0	3 (17)
Respiratory tract infection	1 (6)	0	1 (6)	2 (12)	0	0	3 (17)
Staphylococcal sepsis	1 (6)	0	1 (6)	1 (6)	0	0	3 (17)
Upper respiratory tract infection bacterial	0	0	2 (12)	1 (6)	0	0	3 (17)
Urinary tract infection pseudomonal	1 (6)	1 (6)	3 (18)	0	0	0	3 (17)
Investigations	12 (71)	2 (12)	10 (59)	13 (76)	10 (77)	3 (50)	17 (94)
Antinuclear antibody positive	1 (6)	0	0	0	4 (31)	0	5 (28)

System Organ Class Preferred Term, (data presented as n [%])	Pre-Treatment (N=17)	Treatment (N=17)	3-Month Hospitalization (N=17)	3 Months to 3 Years Follow-up (N=17)	4-7 Years Follow-up (N=13)	≥8 Years Follow-up (N=6)	Total (N=18)
Blood immunoglobulin E increased	0	0	0	3 (18)	3 (23)	0	5 (28)
Hepatic enzyme increased	0	0	4 (24)	2 (12)	0	0	5 (28)
Computerized tomography thorax abnormal	3 (18)	0	0	1 (6)	0	0	4 (22)
Tympanometry abnormal	1 (6)	0	0	2 (12)	1 (8)	0	4 (22)
Blood alkaline phosphatase increased	1 (6)	1 (6)	1 (6)	1 (6)	0	0	3 (17)
Electrophoresis protein abnormal	0	0	1 (6)	2 (12)	0	0	3 (17)
Nuclear magnetic resonance image brain abnormal	3 (18)	0	0	0	0	0	3 (17)
Pulmonary function test abnormal	0	0	0	1 (6)	2 (15)	1 (17)	3 (17)
Weight decreased	0	0	0	2 (12)	1 (8)	0	3 (17)
Skin and subcutaneous tissue disorders	4 (24)	0	7 (41)	10 (59)	5 (38)	2 (33)	16 (89)
Dermatitis atopic	1 (6)	0	0	2 (12)	2 (15)	0	5 (28)
Skin lesion	1 (6)	0	1 (6)	2 (12)	0	0	4 (22)
Dermatitis	0	0	2 (12)	0	1 (8)	0	3 (17)
Rash	0	0	1 (6)	2 (12)	0	0	3 (17)
Blood and lymphatic system disorders	4 (24)	0	11 (65)	8 (47)	2 (15)	0	16 (89)
Anaemia	1 (6)	0	3 (18)	3 (18)	0	0	7 (39)
Neutropenia	0	0	5 (29)	2 (12)	0	0	6 (33)
Eosinophilia	1 (6)	0	1 (6)	2 (12)	0	0	4 (22)
Respiratory, thoracic and mediastinal disorders	7 (41)	0	2 (12)	12 (71)	6 (46)	3 (50)	14 (78)
Cough	1 (6)	0	0	5 (29)	3 (23)	1 (17)	8 (44)
Interstitial lung disease	2 (12)	0	1 (6)	0	0	0	3 (17)
Pneumonitis	2 (12)	0	1 (6)	0	0	0	3 (17)
Productive cough	1 (6)	0	0	1 (6)	1 (8)	0	3 (17)
Gastrointestinal disorders	4 (24)	2 (12)	7 (41)	7 (41)	6 (46)	1 (17)	13 (72)
Diarrhoea	3 (18)	1 (6)	4 (24)	6 (35)	3 (23)	0	10 (56)
Vomiting	2 (12)	1 (6)	1 (6)	1 (6)	1 (8)	0	6 (33)
Enteritis	0	0	1 (6)	1 (6)	1 (8)	1 (17)	3 (17)
General disorders and administration site conditions	6 (35)	3 (18)	1 (6)	9 (53)	5 (38)	0	12 (67)
Pyrexia	4 (24)	1 (6)	1 (6)	6 (35)	4 (31)	0	8 (44)

System Organ Class Preferred Term, (data presented as n [%])	Pre-Treatment (N=17)	Treatment (N=17)	3-Month Hospitali-zation (N=17)	3 Months to 3 Years Follow-up (N=17)	4-7 Years Follow-up (N=13)	≥8 Years Follow-up (N=6)	Total (N=18)
Nervous system disorders	3 (18)	0	0	7 (41)	3 (23)	1 (17)	12 (67)
Cognitive disorder	0	0	0	3 (18)	2 (15)	0	5 (28)
Psychomotor hyperactivity	1 (6)	0	0	2 (12)	0	0	3 (17)
Congenital, familial and genetic disorders	8 (47)	0	0	6 (35)	2 (15)	0	11 (61)
Cryptorchism	3 (18)	0	0	3 (18)	2 (15)	0	6 (33)
Phimosis	2 (12)	0	0	5 (29)	0	0	6 (33)
Hepatobiliary disorders	2 (12)	0	4 (24)	3 (18)	2 (15)	2 (33)	10 (56)
Hepatic steatosis	0	0	1 (6)	0	1 (8)	2 (33)	4 (22)
Hepatomegaly	2 (12)	0	0	1 (6)	0	0	3 (17)
Musculoskeletal and connective tissue disorders	4 (24)	0	0	2 (12)	2 (15)	0	7 (39)
Foot deformity	1 (6)	0	0	0	2 (15)	0	3 (17)
Muscle atrophy	1 (6)	0	0	0	2 (15)	0	3 (17)
Endocrine disorders	3 (18)	0	0	2 (12)	1 (8)	1 (17)	6 (33)
Hypothyroidism	2 (12)	0	0	2 (12)	0	0	4 (22)
Vascular disorders	2 (12)	0	3 (18)	1 (6)	1 (8)	0	6 (33)
Hypertension	1 (6)	0	3 (18)	0	1 (8)	0	5 (28)
Neoplasms[a]	1 (6)	0	1 (6)	1 (6)	1 (8)	3 (50)	5 (28)
Skin papilloma	0	0	0	0	1 (8)	3 (50)	3 (17)

Notes: Subjects are included in the denominator if follow-up is ongoing for the period considered. Subject [redacted] started follow-up at Year 13 but has AEs reported at Year 8 and Year 12, which are included. Subject [redacted] had an AE of Pharyngitis which occurred in the LTFU but the dates were not known so it appears in the Total column only.

a. The neoplasms SOC includes benign, malignant and unspecified, including cysts and polyps.

The majority of these events occurred within the first 35 days post GSK2696273 gene therapy and all were resolved, indicating that they were likely to be a consequence of conditioning with busulfan. Granulocytopenia, thrombocytopenia, and anaemia are known AEs reported with busulfan therapy. Anaemia and neutropenia were considered very common ADR attributed to busulfan. The skin and subcutaneous tissue disorders reported in 3 or more subjects were atopic dermatitis, skin lesion, dermatitis and rash. Dermatitis and rashes are common in children with ADA-SCID and in healthy children. One subject had 2 AEs of skin papilloma, majority of which occurred in the ≥8 year follow-up period and all resolved. Skin papillomas are usually benign tumours caused by the human papilloma virus (HPV) and have been reported in late-onset ADA deficiency and as severe papillomavirus disease after BMT. Cellular immunity including T cell and NK cell cytotoxicity is critical to host defence against HPV.

Adverse events by maximum grade

Summary of adverse events by severity (all studies and CUP safety population)

Toxicity Grade, (data presented as n [%])	Pre-treatment (n=17)	Treatment (n=17)	3-month hospitali-zation (n=17)	3 months to 3 years follow-up (n=17)	4-7 years follow-up (n=13)	≥8 years follow-up (n=6)	Total (n=18)
Grade 1	14 (82)	5 (29)	16 (94)	17 (100)	13 (100)	6 (100)	18 (100)
Grade 2	6 (35)	2 (12)	13 (76)	15 (88)	9 (69)	5 (83)	17 (94)
Grade 3	2 (12)	0	8 (47)	10 (59)	5 (38)	2 (33)	15 (83)
Grade 4	0	0	5 (29)	1 (6)	0	0	5 (28)
Missing	6 (35)	1 (6)	0	0	0	0	6 (33)

Notes: Subjects are included in the denominator if follow-up is ongoing for the period considered. Subject [redacted] started follow-up at Year 13 but has AEs reported at Year 8 and Year 12, which are included. Subject [redacted] had an AE of Pharyngitis which occurred in the LTFU but the dates were not known so it appears in the Total column only.

More than half of subjects reported grade 3 (severe) infection AEs and most of these occurred in the 3-month to 3-year follow-up phase. The most frequently reported grade 3 AE was device-related infection (5 subjects). Other frequently reported (>2 subjects) SAEs were hepatic enzyme increased, anaemia, neutropenia, gastroenteritis, pneumonia, and varicella. All of the most frequently reported SAEs resolved. Three of the grade 3 neurologic/cognitive/hearing AEs were ongoing at the time of dossier submission. Five subjects (28%) had grade 4 events and most events occurred during the 3-month hospitalisation phase. Five grade 4 AEs were reported in the SOC of blood and lymphatic system disorders. All of the grade 4 blood and lymphatic disorder AEs occurred in the 3-month hospitalisation phase and the majority occurred within 35 days of gene therapy, suggesting that events may be related to busulfan. Two subjects had grade 4 infections and furthermore, six subjects had AEs with missing reported severity. All of these events were reported in the pre-treatment phase, with the exception of one event of vomiting reported in the treatment phase.

Treatment-related adverse events

Adverse drug reactions with GSK2696273 gene therapy

MedDRA System Organ Class	Very common	Common
Blood and Lymphatic System Disorders	Anaemia[a] Neutropenia[a]	Autoimmune haemolytic anaemia; Autoimmune aplastic anaemia; Autoimmune thrombocytopenia
Endocrine Disorders	Hypothyroidism	Autoimmune thyroiditis
General Disorders and Administration Site Conditions	Pyrexia	--
Hepatobiliary Disorders	--	Autoimmune hepatitis
Investigations	Hepatic enzyme increased[a]	
Nervous System Disorders	--	Guillain-BarréGuillain-Barré syndrome
Respiratory, Thoracic, and Mediastinal Disorders	Rhinitis allergic, asthma	--
Skin and Subcutaneous Tissue Disorders	Dermatitis atopic, eczema	--
Vascular Disorders	Hypertension[a]	--

a. ADRs considered potentially related to busulfan conditioning

System Organ Classes with the most frequently reported AEs were infections and infestations, investigations, blood and lymphatic system disorders, and skin and subcutaneous tissue disorders. For each of these, the AE density as estimated by exposure adjusted incidence of events per 100 patient years, for the SOC was greatest during a phase before, pre-treatment phase, or during hospitalisation, treatment and 3- month hospitalization phases, compared with the AE densities recorded for follow-up phases beginning 3 months post GSK2696273 gene therapy and later. The very common ADRs were anaemia, asthma, dermatitis atopic, eczema, hypothyroidism, hepatic enzyme increased, hypertension, neutropenia, pyrexia, and rhinitis allergic. ADRs considered to be possibly related to busulfan conditioning included anaemia, neutropenia, elevations in liver transasminases, and hypertension. The use of busulphan hampers a clear assessment of the product safety.

In general, it seems that the incidence of AE decreased over time and that the most AEs were infrequent beyond year 3, which is reassuring. In addition, most of AEs were grade 1 and 2 in severity. However, "Infections", "Investigations" and "Nervous system disorders" continued being reported beyond 3 years suggesting that close monitoring of patients is necessary in the long-term. With regard to treatment-related AEs, only 2 events were judged as treatment related by investigators. The AEs reflected in the SmPC are the result of the review of the integrated safety data done by applicant as post-hoc analyses where a number of criteria were considered to classify the AEs. It is, however, acknowledged that there are difficulties to establish a firm association of the AEs with this investigational product and in this particular setting.

Adverse reactions potentially related to immune reconstitution

In the integrated population, the following autoimmune events were reported for subjects post-gene therapy: hypothyroidism, autoimmune thyroiditis, autoimmune thrombocytopenia, autoimmune haemolytic anaemia, autoimmune aplastic anaemia, Guillain-Barré syndrome, autoimmune hepatitis, antinuclear antibody (ANA) test positive, anti-thyroid antibody positive, anti-smooth muscle antibody positive, anti-neutrophil cytoplasmic antibody positive, and Coombs direct test positive, see table below.

Subjects with AEs potentially related to autoimmunity by study phase

Preferred Terms	Pre-treatment (n=17)	Treatment (n=17)	3-month Hosp (n=17)	3-months to 3-years Follow-up (n=17)			4-7 years Follow-up (n=13)			≥8 years Follow-up (n=6)
				3 mo-1 yr	1 yr-2 yrs	2 yrs-<4 yrs	4 yrs-5 yrs	5 yrs-6 yrs	6 yrs-<8 yrs	
Anti-nuclear antibody test positive	■						■	■	■	■
Coombs direct test positive								■		
Anti-smooth muscle antibody positive							■	■		
Anti-neutrophil cytoplasmic antibody									■	
Anti-thyroid antibody positive			■							
Autoimmune aplastic anaemia/aplastic anaemia						■	■			
Autoimmune haemolytic anaemia	■					■	■			
Autoimmune neutropenia[a]			■							
Autoimmune hepatitis						■				
Autoimmune thrombocytopenia			■	■	■					
Guillain-Barré syndrome						■				
Hypothyroidism/ Autoimmune thyroiditis	■			■		■				■

Abbreviations: ANA = antinuclear antibody; ANCA = anti-neutrophil cytoplasmic antibody; ASMA = anti-smooth muscle antibody; hosp = hospitalization; mo = month; yr = year; yrs = years

The majority of the events post-gene therapy occurred within 3 years of treatment with GSK2696273. Most resolved, with the exception of hypothyroidism (4 subjects), ANA test positive (3 subjects), and autoimmune haemolytic anaemia and autoimmune thrombocytopenia. As the nature and timing of the events are consistent with autoimmune events observed during immune reconstitution of SCID patients with other therapies, the applicant considers that these events are adverse reactions and could be a result of the immune reconstitution provided by GSK2696273 and/or possibly due to immune dysregulation inherent in ADA-SCID. ANA-positivity was reported in 6 subjects (1 during the pre-treatment phase, 4 during the 4-7 year follow-up period and 1 during the >8 year follow-up period). A positive anti-thyroid antibody occurred in 1 subject who was not described as having an abnormal thyroid and this was not considered a clinically relevant finding. Positivity of anti-smooth muscle antibody, anti-neutrophil cytoplasmic antibody, and Coombs direct test are considered useful supportive tests of clinical findings, but were not clinically relevant on their own to warrant labelling as ADR. In the integrated population, 5 subjects reported a total of 7 events of blood IgE increased and 4 subjects reported a total of 3 events of eosinophilia. In this subgroup of subjects, multiple clinical allergy AEs overlapped with the IgE and eosinophilia events: dermatitis atopic (5 events in 5 subjects), eczema/eczema nummular (4 events in 3 subjects), rhinitis allergic/rhinorrhoea (7 events in 4 subjects), and asthma (2 events in 2 subjects). The majority of events were reported during the 3 month to 3-year follow-up phase. As with autoimmunity, the nature and timing of the events are consistent with such events reported in the literature during immune reconstitution. The applicant considers that these events could be a result of the immune reconstitution provided by GSK2696273 gene therapy and/or possibly due to immune dysregulation inherent in ADA-SCID. Allergy related events were classified as adverse reactions. Subjects who were described as having 'eczema nummular' and 'rhinorrhoea' were considered to be adequately described by the terms 'eczema' and 'rhinitis allergic', respectively. Pyrexia was reported in 8 subjects and occurred both pre- and post-treatment and the applicant considers pyrexia to be a very common ADR.

It should be noted that the literature refers to the fact that immunodeficiency and immune dysregulation, which often present as multiple forms of autoimmunity, may occur concurrently in the same patient, and represent a breakdown or inadequate development of immune tolerance. In this

respect, autoimmunity has been reported in SCID patients who achieve partial or poor immune reconstitution after BMT or with PEG-ADA treatment and in late onset ADA deficiency. This can manifest itself as haemolytic anaemia, hypothyroidism, immune thrombocytopenia, or atopy. Reports of elevation in IgE levels, eosinophilia, asthma, and allergies have also been described in delayed-onset ADA-SCID. While it may be reasonable to include neutropenia, but not autoimmune neutropenia, as a very common ADR that is considered potentially related to busulfan, the SmPC has been updated during the assessment and "antinuclear antibody positive", "anti-neutrophil cytoplasmic antibody positive", and "smooth muscle antibody positive" findings have been added as adverse reactions to section 4.8. In addition, "hypothyroidism" and "autoimmune thyroiditis" are named in the tabulated list of the SmPC. The main concern is that the events occurred beyond 1 year and in many cases 3 years after gene therapy, when the immune reconstitution. The risk of therapeutic failure exists and may be high. Therefore long-term monitoring of immunogenicity in the registry was requested by the CAT/CHMP.

Serious adverse event/deaths/other significant events

Serious adverse events

In the integrated database, 15 of 18 subjects have reported 39 SAEs post-treatment, see table below.

All Subjects with SAEs Post-treatment in the GSK2696273 Clinical Programme

Subject	Gender	SAE (preferred term)	Study phase/Year	Onset* (days)	Duration (days)	Outcome	Maximum toxicity grade
[redacted]		Urinary tract infection	Year 13	4867	4	Resolved	Grade 2
		Pancytopenia	3-month hospitalization	29	22	Resolved	Grade 3
		Pneumonia	Year 10	3677	8	Resolved	Grade 3
		Varicella	Year 6	2066	8	Resolved	Grade 3
		Varicella	3-month to 3 year follow-up	692	6	Resolved	Grade 3
		Device-related infection	3-month hospitalization	81	12	Resolved	Grade 3
		Epstein-Barr virus infection	3-month hospitalization	84	9	Resolved	Grade 3
		Device-related infection	3-month to 3 year follow-up	406	4	Resolved	Grade 3
		Autoimmune hepatitis	3-month to 3-year follow-up	755	14	Resolved	Grade 3
		Aplastic anaemia	3-month to 3 year follow-up	1145	120	Resolved	Grade 3
		Autoimmune aplastic anaemia	Year 5	1665	10	Resolved	Grade 3
		Hypertension	3-month hospitalization	37	75	Resolved	Grade 3
		Neutropenia	3-month hospitalization	46	66	Resolved	Grade 3
		Autoimmune thrombocytopenia	3-month hospitalization	134	12	Resolved	Grade 3
		Autoimmune thrombocytopenia	3-month to 3-year follow-up	353	16	Resolved	Grade 3
		Urinary tract infection	3-month to 3-year follow-up	398	10	Resolved	Grade 3
		Upper respiratory tract infection bacterial	3-month to 3-year follow-up	599	12	Resolved	Grade 3
		Device-related infection	3-month to 3-year follow-up	638	14	Resolved	Grade 3
		Gastroenteritis	3-month to 3-year follow-up	512	6	Resolved	Grade 3
		Guillain-Barré syndrome	3-month to 3-year follow-up	1125	4	Resolved	Grade 3
		Device-related infection	3-month to 3-year follow-up	148	11	Resolved	Grade 3
		Device-related infection	3-month to 3-year follow-up	331	10	Resolved	Grade 3
		Device-related infection	3-month to 3-year follow-up	374	17	Resolved	Grade 3
		Device-related infection	3-month hospitalization	125	16	Resolved	Grade 3
		Pneumonia	Year 4	1603	10	Resolved	Grade 3
		Lipofibroma	3-month to 3-year follow-up	1154	4	Resolved	Grade 3

Subject	Gender	SAE (preferred term)	Study phase	Onset[a]	Duration	Outcome	Maximum toxicity grade
[redacted]		Device-related infection	3-year follow-up	378 days	9 days	Resolved	Grade 3
		Gastroenteritis	3-year follow-up	475 days	7 days	Resolved	Grade 3
		Diarrhoea	3-year follow-up	533 days	9 days	Resolved	Grade 3
		Pyrexia	3-year follow-up	680 days	4 days	Resolved	Grade 3
		Pyoderma	Year 4	1443 days	20 days	Resolved	Grade 3
		Pyrexia	Year 4	1602 days	2 days	Resolved	Grade 2
		Thermal burn	Year 5	1813 days	110 days	Resolved with sequelae	Grade 2
		Neutropenia[b]	3-month hospitalization	46 days	29 days	Resolved	Grade 4
		Respiratory tract infection	3-year follow-up	242 days	3 days	Resolved	Grade 3
		Staphylococcal sepsis	3-year follow-up	474 days	9 days	Resolved	Grade 4
		Pneumonia	3-month hospitalization	106 days	46 days	Resolved	Grade 4
		Gastroenteritis	3-year follow-up	250 days	28 days	Resolved	Grade 2
		Meningitis	3-year follow-up	261 days	17 days	Resolved	Grade 3

Abbreviations: NA = not applicable; SAE = serious adverse event.
a. Time to onset since administration of GSK2696273.
b. Neutropenia in Subject [redacted] was identified as an event potentially related to autoimmunity (verbatim term: prolonged neutropenia (anti-neutrophils antibodies positive)).

Fourteen of the SAEs were reported in 2 subjects. One subject was withdrawn due to reasons related to unsuccessful response to gene therapy. All SAEs reported as of the integrated data cut-off resolved. Twenty-four of the 39 SAEs reported post-treatment in the integrated dataset were infections, all of which resolved. The most frequently reported SAEs (>3 subjects) were in the SOC of infections and infestations. Three of the SAEs were grade 4 (neutropenia, pneumonia, and Staphylococcal sepsis):

- Neutropenia: In one subject, prolonged neutropenia developed approximately 6 weeks following gene therapy and was treated with G-CSF, high dose IVIG, and prophylactic antibiotics. The neutropenia was considered to be due to a combination of the effects of the conditioning regimen and the presence of anti-neutrophil antibodies. This SAE resolved in 29 days.
- Staphylococcal sepsis: One subject had a Grade 4 SAE of Staphylococcal sepsis with onset 1.3 years after GSK2696273 treatment. The subject had a CVC in place at the time of this event. It was treated with vancomycin, ceftazidime, and acyclovir. The event was considered not related to gene therapy, per the investigator, and resolved after 9 days.
- Pneumonia: One subject had a grade 4 SAE of pneumonia with onset 3.5 months after gene therapy. The subject received the following concomitant medications to treat pneumonia: cefpodoxime, amoxicillin/clavulanate, ceftriaxone, clarithromycin, piperacillin/tazobactam, sulfamethoxazole/trimethoprim, voriconazole, salbutamol and budesonide. In addition, the subject received an infusion of autologous CD34- cells due to persistent lymphopenia. This subject also received PEG-ADA on 3 occasions while the pneumonia SAE was ongoing.

Other frequently reported SAEs were autoimmune manifestations, with 4 subjects reporting 6 events (anti-neutrophil antibody-induced neutropenia, autoimmune thrombocytopenia, autoimmune aplastic anaemia, autoimmune hepatitis, and Guillain-Barré syndrome). The subject with 2 events of autoimmune thrombocytopenia had a history of autoimmune manifestations and was subsequently withdrawn due to unsuccessful response to gene therapy. One subject had an SAE of hypertension and required long-term therapy with amlodipine. Serious opportunistic infections are often observed in patients with immunodeficiencies, but were not common in the GSK2696273 clinical programme. Other opportunistic infections reported in more than 1 subject in the GSK2696273 clinical programme included Candida, Clostridium difficile, Varicella, and Aspergillus. Four subjects experienced Varicella infections; 2 subjects had grade 3 Varicella infections that were SAEs. All of these subjects recovered from the serious infection. None of the SAEs were considered related to the product.

Adverse events were reported in the GSK2696273 clinical programme that required administration of PEG-ADA, high-dose IVIG, high-dose steroids, or additional cells, i.e., back-up bone marrow, un-manipulated CD34+ cells, or CD34- fraction), see table below.

Adverse events leading to administration of PEG-ADA, high-dose IVIG, high-dose steroids, or additional cells (All studies and CUP safety populations)

Subject number	Event	Study phase	Outcome	Intervention[a, b]
[redacted]	Pancytopenia	3-month hosp	Resolved	GM-CSF Cryopreserved autologous unmanipulated bone marrow cells (1.42 x 10^6 CD34+/kg and 10.7 x 10^4 CFU-gm/kg)
	Epstein-Barr virus infection	3-month hosp	Resolved	Cryopreserved autologous lymphocytes (1.29 x 10^9 cells, CD34- fraction of BM harvest) Rituximab (mAb anti-CD20)
	Autoimmune hepatitis	3 year follow-up	Resolved	IV solumedrol, prednisone po PEG-ADA
	Autoimmune aplastic anaemia (>1 hospitalisation)	3-year follow-up and Year 5	Resolved	Prednisone, rituximab, IV immunoglobulins, RBC transfusion
	Hypertension[b]	3-month hosp	Resolved	Furosemide, nifedipine, amlodipine, ramipril
	Neutropenia	3-month hosp	Resolved	G-CSF, IV immunoglobulins, filgrastim, "Mielograstim", lenograstim, periodic RBC transfusions[c]
	Autoimmune thrombocytopenia (>1 hospitalization)	3-month hosp	Resolved	IV methylprednisolone, prednisone po IV immunoglobulins, irradiated platelet transfusions
	Anaphylactic reaction to IVIG	3-year follow-up	Resolved	IV prednisone
	Guillain-Barré syndrome	3-year follow-up	Resolved	IV immunoglobulins
	Lipofibroma[b]	3-year follow-up	Resolved	Surgical resection of abdominal lipofibroma
	Anaemia	3-year follow-up	Resolved	RBC transfusion
	Neutropenia	3-month hosp	Resolved	G-CSF
	Pneumonia	3-month hosp	Resolved	PEG-ADA, IV immunoglobulins
	CMV reactivation	3-month hosp	Resolved	CD34- cell fraction (i.e., cells remaining after CD34+ purification)
	Lymphopenia	3-month hosp	Resolved	
	Meningitis	3-year follow-up	Resolved	Dexamethasone; PEG-ADA
	Neutropenia	3-month hosp	Resolved	G-CSF

Abbreviations: BM = bone marrow; CD34 –haematopoietic stem progenitor cells; CFU = colony forming units; CMV = cytomegalovirus; CUP = compassionate use programme; G-CSF = granulocyte colony stimulating factor; GM-CSF – granulocyte-macrophage colony-stimulating factor; hosp = hospitalization; IV = intravenous; IVIG = intravenous immunoglobulin; PEG-ADA = polyethylene glycol adenosine deaminase; po = oral; RBC = red blood cell; SAE = serious adverse event

a. Only the following interventions are listed, as reported in the SAE narratives: PEG-ADA, high-dose IVIG, high-dose steroids, or additional cells (back-up bone marrow, unmanipulated CD34+ cells, or CD34- fraction (see Section 2.2). Subjects may have received other medications (e.g., antibiotics), per standard-of-care, that are not listed. Where IVIG is listed, it represents high-dose, as opposed to monthly maintenance. Based on the methodology of data collection, the listing represents events post-gene therapy.

b. Although interventions were not PEG-ADA, high-dose IVIG, high-dose steroids, or additional cells, hypertension in Subject [redacted] and lipofibroma in Subject [redacted] are reported here due to substantial concomitant therapy or intervention (i.e., surgery).

c. Periodic RBC transfusions were administered to maintain an adequate concentration of hemoglobin and to reduce bone marrow erythropoietic drive to favour the myeloid differentiation.

In addition, substantial intervention was required for all cases of unsuccessful response to gene therapy, poor immune reconstitution, premature withdrawals, and in 2 subjects due to contamination of the drug product.

Adverse events of interest

Based on the expected events in patients with ADA-SCID, the risks of gene therapy, regulatory guidance, and the reporting of treatment-related AEs as assessed by the investigator, the following events were identified as AEs of special interest:

Infections: Infections of the respiratory tract (upper respiratory tract, rhinitis, bronchitis, nasopharyngitis, pneumonia, and sinusitis) were the most frequently reported AEs. They were of limited duration and most were not serious. These types of infections are prevalent in ADA-SCID and in the normal paediatric populations. The densities were highest in the pre-treatment to 3-month hospitalisation period and they decreased with time. A total of 9 subjects reported ear infection and/or otitis media, with onset mostly in the pre-treatment to the 3-year follow-up period. Four subjects experienced varicella infections and all of them recovered. Other infections that are generally considered important in an immune-compromised population were not common, occurred predominantly early after gene therapy, and resolved. Twenty four of the 39 SAEs reported post-treatment in the integrated dataset were infections, all of which resolved. In the overall integrated population for safety (without censoring of subjects requiring intervention), 22 infections were classified as "severe" per the clinical programme definition.

Urinary tract infections (UTIs) were of the most commonly reported infections in this cohort of ADA-SCID subjects. The number of UTIs was higher than might be expected relative to the general paediatric population, but several did occur in 2 subjects considered to have had an unsuccessful response to GSK2696273 treatment, and in others with congenital abnormalities (phimosis, crytochordism, and ambiguous genitalia) known to cause a predisposition for UTIs.

Neurologic, central nervous system, and hearing adverse events: 17 of the 18 subjects in the GSK2696273 programme had these events during treatment or post-treatment, and many subjects reported events pre-treatment. The most frequently reported event was cognitive disorders (5 subjects). The other events reported in more than 1 subject were deafness, bilateral deafness and psychomotor hyperactivity. A standardized MedDRA query for hearing impairment identified 9 subjects with 12 AEs during treatment or post-treatment, with the median time to onset being 2.90 years (range 0.16-12.47). Fourteen of the 17 subjects with neurologic, CNS, or hearing events on or after gene therapy had either relevant conditions ongoing at screening or events during the pre-treatment phase. Nine of these 10 subjects were on PEG-ADA prior to gene therapy. It is noteworthy that parental consanguinity was reported in 9 of 18 subjects. The literature review refers to sensori-neural deafness and neurological and behavioral abnormalities among patients treated with BMT, ERT, and gene therapy. In this respect there is similarity in the neurological events present at baseline and observed in LTFU throughout to those observed in patients treated with BMT. It would therefore appear that gene therapy does not seem to prevent the appearance of these events.

Immune reactions: For GSK2696273, there has been no immunogenicity testing conducted to date. This approach was taken because GSK2696273 was considered to have low risk for immunogenicity in ADA-SCID patients. This low risk is due to contributing factors such as the nature of this disease (immune deficiency), the attributes of the therapy (busulfan preconditioning and IVIG and autologous-derived cells for GSK269273), the single administration of GSK269723, and the intracellular location of ADA enzyme precluding it from extracellular antibody recognition. Most subjects had been on PEG-ADA prior to gene therapy and although the level of anti- PEG-ADA antibodies was not assessed in the clinical studies with GSK2696273, there was no evidence of a loss of enzymatic function in any subject. A customised search of AE listings for systemic allergic events (angioedema, anaphylactic reaction, and severe cutaneous adverse reactions) that could be potentially related to immunogenicity did not

identify any such events within the first year of GSK2696273 administration. All AEs potentially related to autoimmunity have been adequately described in the SmPC.

The CAT/CHMP noted that the applicant made assumptions regarding the low risk for immunogenicity of Strimvelis and hence, did not evaluate the anti-ADA antibodies. This is considered of interest, since the majority of patients on PEG-ADA do develop antibodies, and in 10% of patients, these are neutralising. More importantly these can cross-react with the human ADA. It is acknowledged that human ADA is intra-cellular and would be unlikely to be affected, however, firm proof of this has not been provided and thus, in those patients who showed an unsuccessful response to gene therapy, particularly early on after treatment, PEG-ADA antibodies would have facilitated interpretation of the data. In this respect the applicant has presented a detailed argument against the possibility of antibodies to PEG-ADA causing clinically significant and treatment altering effects especially with respect to those patients who had treatment failure within a few months after receiving gene therapy. While it is acknowledged that the applicant has highlighted significant points in favour of a lack of clinically significant effects of antibodies, this cannot be ruled out in some cases. Therefore, the CAT/CHMP requested the applicant to evaluate immunogenicity in a post-marketing setting and this is included in the RMP. The AEs of systemic allergic events (angioedema, anaphylactic reaction, and severe cutaneous adverse reactions) that could be potentially related to immunogenicity will also be monitored during LTFU via the registry, which the applicant committed to.

Oncogenesis: In the GSK2696273 programme, no malignancies were reported, as shown by the SMQ for malignant tumours. The MedDRA SMQ for tumours of unspecified malignancy revealed 1 event of pulmonary mass. Additional AE terms that could indicate leukemic transformation were investigated, including hyperimmunglobinaemia and serum protein electrophoresis abnormal. Hyperimmunoglobinaemia could occur as a result of inappropriate expansion of a B-cell clone and could be a marker of leukemic transformation. An abnormality in the serum protein electrophoresis could also indicate a monoclonal gammopathy, which is a sign of expansion of a particular B-cell clone and could signal leukaemic transformation. One subject reported an AE of immunoglobulins increased in the 3-month to 3-year follow-up phase; the event resolved over approximately 3 years. At the time of this event, the subject also reported AEs of eosinophilia, electrophoresis protein abnormal, and NK cell count increased; all of these events resolved.

Another important signal of leukemic transformation is the presence of a skewed T-cell V beta repertoire where only a few V beta chains are present, which would indicate the expansion of a single clone of T-cells. All subjects had evidence of a polyclonal T-cell V beta repertoire with more than a single V beta family represented. In most subjects, bone marrow analyses by morphology of the aspirate showed the presence of lymphocyte, myeloid and erythroid developing cells and bone marrow immunophenotype showed the presence of B-cells, T-cells, and NK cells. Cytogenetic analyses (karyotype) were normal in the bone marrow and peripheral blood; no abnormal blast values were found in either peripheral blood or bone marrow and therefore, no AEs were reported.

Retroviral insertion site (RSI) analysis: Retroviral insertion site analysis was conducted in 14 subjects in the AD1115611 LTFU study. The recovered RIS were presented to allow identification of sites with high relative clonal abundance (>10%). The proximity of these sites to cancer-related genes was mapped with particular focus on genes previously associated with leukaemia or MDS in clinical trials of similar retroviral vectors. A total of 2333 unique insertion sites were mapped. Insertion sites present at >10% abundance were mapped against the nearest genes, both up and downstream on coding and non-coding. As would be expected for a retroviral vector that inserts semi-randomly into the genome, all but 1 subject had insertion sites close to genes linked with cancer. The main concern with this particular type of vectors is insertional mutagenesis with the potential for clonal expansion and

neoplasia. However, it is not entirely clear that despite the existing evidence in other diseases like SCID-X, WAS and CGD, none of 18 subjects appear to have developed leukaemia or MDS during a median of 7 years and a maximum of 13 years of follow-up. In addition, investigations for other haematological abnormalities that could be indicative of clonal abnormalities including B cell immunoglobulin production, T-cell receptor V-beta repertoire, bone marrow morphology and immunophenotype, peripheral blood smears, and cytogenetic karyotype analysis did not appear to show clinically significant changes. As ADA is an enzyme, and is not involved in processes controlling cell proliferation/apoptosis, tight gene expression regulation has not yet shown to be crucial. Overexpression is unlikely to cause significant side effects. ADA is known as a metabolic "house-keeping" protein that is constitutively expressed in all cell types. The applicant stated that estimates of clonal abundance and comprehensive identification of integration site locations using current methods are compromised by several types of recovery biases and sources of variability and therefore, RSI is currently unsuitable as an individual subject monitoring safety tool. However, the applicant evaluated RSI analysis at a single point in time and has raised the issue of assay variability. To test the persistence and contribution of identical insertion sites in a given cell lineage, patients should be studied at different time points after gene therapy. In response to this request by the CAT/CHMP, the applicant summarised new data that supports demonstration of polyclonality and also summarised plans for investigating new and potentially more robust methods for RIS analysis. An explanation of why RIS analysis is not a suitable prospective test for lymphoproliferative SAE detection was also provided along with a summary of the measures the applicant is proposing in the post-approval safety study to monitor for oncogenesis events. This was considered sufficient by the CAT/CHMP and the measure for monitoring genotoxicity in the post-marketing setting has been agreed and included in the RMP.

There is a considerable effort to improve RIS detection that might be modified following new technical developments. Nevertheless, it cannot be assumed that adverse events will not occur and accordingly, patient monitoring needs to be continued to ensure the long term safety of gene therapy. Although the CAT/CHMP recommended lifelong monitoring, the applicant has proposed a 15 year period and provided a reasonable justification for this duration. This includes a proposed registry with follow up of 50 patients for a minimum of 15 years. In this respect, the first 4 patients from the trials who currently have approximately 15 years of follow up would be followed up for a period of 33 years by the time the registry will have closed. In addition, the applicant proposes to continue to solicit information on malignancy/death/adverse pregnancy outcome every 2 years past a subject's 15-year post-gene therapy anniversary until the registry is complete. Furthermore, if any relevant efficacy or emergent safety concerns arise during one of the interim study assessments, the applicant would seek a consultation with the regulatory authorities to discuss whether the duration of the registry should be extended beyond the current 15-year proposal. This is deemed acceptable to the CAT/CHMP. The AEs reported with respect to decreased T-cell V beta repertoire, pulmonary mass, lipofibroma, electrophoresis protein abnormal as well as skin papilloma, have been adequately reflected in the SmPC.

Hepatic laboratory abnormalities and hepatobiliary events: Thirteen subjects reported 40 hepatobiliary AEs, including hepatic laboratory abnormalities during the GSK2696273 clinical programme. Six subjects had hepatobiliary conditions at screening or pre-treatment events. Half of the events (21/40) reported at any time during pre- or post- GSK2696273 therapy were related to elevations in liver function tests (liver transaminases or alkaline phosphatase), most were >3 times the upper limit of normal (ULN) but <5 times ULN, and occurred predominantly during the 3-month hospitalisation phase, post-busulfan. Approximately half of the subjects (7/13) with elevations in liver function tests had other hepatobiliary disorders and all of the elevations in liver function test resolved without

intervention. Busulfan is known to cause elevations in transaminases and hepatic veno-occlusive disease. Autoimmune hepatitis is considered an ADR for GSK2696273 and hepatic enzyme increased considered an ADR attributed to busulfan. It is acknowledged that busulphan can adversely affect hepatic enzymes, while autoimmune hepatitis can be considered an ADR for GSK2696273, however, the occurrence of 4 cases of hepatic steatosis during LTFU needs to be kept under review which has been satisfactorily addressed in the long term follow up via the registry requested by the CAT/CHMP.

Deaths

No deaths have been reported during the GSK2696273 clinical programme.

Laboratory findings

Haematology

Following an initial decrease between baseline and Day 14 consistent with the non-myeloablative conditioning phase, haematology endpoints of haemoglobin, haematocrit, platelet count, and white blood cells (WBC) showed an increase toward pre-treatment values over 3 months to 1 year. Only WBC counts increased above pre-treatment values, which is to be expected as increases in lymphocyte counts occur as immune reconstitution progresses post-gene therapy.

Clinical chemistry and urinalysis

Most clinical chemistry and urinalysis values remained within the normal laboratory ranges. Out-of-range values were noted, but all were temporary and no consistent pattern of clinical concern was observed for any test. Abnormalities or out-of-range values were Changes do not appear to be significantly remarkable.

Safety in special populations

Not applicable to this type of population.

Safety related to drug-drug interactions and other interactions

As GSK2696273 is designed to result in the production of intracellular ADA, which is a protein normally found within the body, it is unlikely to interact with any particular medication. However, no formal interaction studies were performed. This is acceptable to the CAT/CHMP.

Discontinuation due to adverse events

There were no AEs leading to withdrawal. Despite the fact that patients had AEs and SAEs, patients were only withdrawn for unsuccessful gene therapy.

Post marketing experience

There is no post-marketing experience with Strimvelis.

2.6.1. Discussion on clinical safety

Strimvelis safety population was essentially the efficacy population except that two patients started treatment after the data cut-off, however, only SAEs have been reported for these two patients. The dose of busulphan was significantly less than that used in a myeloablative regimen and therefore the AEs observed may not be entirely consistent with its typical safety profile. One patient had an unsuccessful response to gene therapy following treatment with and without conditioning treatment with busulphan. System Organ Classes with the most frequently reported AEs were infections and infestations, investigations, blood and lymphatic system disorders, and skin and subcutaneous tissue

disorders. The very common ADRs were anaemia, asthma, dermatitis atopic, eczema, hypothyroidism, hepatic enzyme increased, hypertension, neutropenia, pyrexia, and rhinitis allergic. ADRs considered to be possibly related to busulfan conditioning included anaemia, neutropenia, elevations in liver transasminases, and hypertension. The use of busulphan limits a clear assessment of the product's safety; however, as already stated, the dose used was much lower than specified. The literature refers to the fact that immunodeficiency and immune dysregulation may occur concurrently in the same patient, and represent a breakdown or inadequate development of immune tolerance. In this respect, autoimmunity has been reported in SCID patients who achieve partial or poor immune reconstitution after BMT or with PEG-ADA treatment and in late onset ADA deficiency. The SmPC has been updated and treatment related AEs such as "antinuclear antibody positive", "antineutrophil cytoplasmic antibody positive", and "smooth muscle antibody positive" have been incorporated as adverse reaction in section 4.8 of the SmPC.
Serious adverse events were reported in most subjects and infections were the most frequent SAEs. Serious opportunistic infections are often observed in patients with immunodeficiencies, but were not common in the GSK2696273 clinical programme. Other opportunistic infections included Candida, Clostridium difficile, Varicella, and Aspergillus infections. All of the affected subjects recovered. None of the SAEs were considered related to the product administration. The majority of infections were reported in the 3 month to 3 year period, many being related to the CVC. With respect to CNS events, the literature refers to sensori-neural deafness and neurological and behavioural abnormalities among patients treated with BMT, ERT, and gene therapy. In this respect, there is similarity in the neurological events present at baseline and observed in LTFU throughout, to those observed in patients treated with BMT. It would appear BMT does not seem to prevent the appearance of these events. As far as immunogenicity is concerned, the anti-ADA antibodies have not been evaluated. This is questionable since the majority of patients on PEG-ADA develop antibodies, and in 10%, these are neutralising. More importantly, they could potentially cross-react with human ADA. It is acknowledged that human ADA is intra-cellular and would be unlikely to be affected; however, a firm proof of this concept has not been provided. Thus, the CAT/CHMP requested the applicant to follow up on these events in the post-marketing setting and this has been agreed.
With respect to hepatic adverse events, it is acknowledged that busulphan can adversely affect hepatic enzymes, while autoimmune hepatitis can be considered as an ADR for GSK2696273; however, the occurrence of 4 cases of hepatic steatosis during LTFU needs to be kept under review and the monitoring of hepatotoxicity is now included in the long-term registry as requested by the CAT/CHMP. Laboratory values for haematology and clinical chemistry do not appear to be significantly remarkable. Adverse events with respect to age and gender in comparison to the integrated population appeared to be generally similar. Furthermore, the applicant will also conduct a post-approval methodology study to investigate the retroviral insertion. It cannot be assumed that related adverse events will not occur and hence, patient monitoring needs to be continued to ensure the long term safety of gene therapy. The CAT/CHMP recommended the genotoxicity and immunogenicity follow up. These have been included in the RMP.
The CAT/CHMP considered that a thorough follow up of patients via registry is an important post-marketing measure and requested a set-up of a registry. All patients will be monitored for immunogenicity, insertional mutagenesis and oncogenesis as well as hepatic toxicity. The applicant will review the occurrence of angioedema, anaphylactic reactions, systemic allergic events and severe cutaneous adverse reactions during the follow up period, particularly in those patients who had unsuccessful response and received ERT or SCT. The applicant will also evaluate intervention-free survival.
In addition, the applicant will conduct a post approval methodology study to investigate the retroviral insertion site analysis.

These measures are considered adequate by the CAT/CHMP. From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

While the short term safety evaluation appears to be hampered by the busulphan conditioning, medium and longer term safety seem to be consistent with safety findings in ADA patients undergoing immune reconstitution. Immunogenicity has not been fully addressed in the clinical programme, especially with respect to antibodies to PEG-ADA, and the effect of inhibitory antibodies with respect to treatment failure cannot be excluded in some cases. Accordingly, the applicant will monitor immunogenicity in new patients entered in the postmarketing registry. In this respect antibodies to PEG-ADA will be evaluated initially, and in those patients in whom inhibitory antibodies are detected, immunogenicity with respect to bovine ADA and human ADA will also be evaluated in a post-marketing setting. The applicant will develop an appropriate screening, confirmatory assays which would have been fully validated for this purpose. Although the main safety concern with respect to oncogenesis and insertional mutagenesis at this point in time does not give rise to major concerns, this cannot be taken for granted and requires further monitoring. Hence, the applicant will conduct a post approval methodology study to investigate the retroviral insertion site analysis.

The CAT considers the following measures necessary to address issues related to safety:

- Non-interventional PASS: In order to investigate the long term safety and efficacy of Strimvelis gene therapy, the MAH should conduct and submit the results of a long term prospective, non-interventional follow up study using data from a registry of patients with adenosine deaminase severe combined immunodeficiency (ADA-SCID) treated with Strimvelis. The MAH will follow up on the risk of immunogenicity, insertional mutagenesis and oncogenesis as well as hepatic toxicity. The MAH will review the occurrence of angioedema, anaphylactic reactions, systemic allergic events and severe cutaneous adverse reactions during the FU period, particularly in those patients who had unsuccessful response and received ERT or SCT. The MAH will also evaluate intervention-free survival.
- The applicant will provide the final study report of the long term follow up study AD1115611 LTFU as an obligation. In this respect all 18 patients should be followed up for a period of 8 years, Category 3, RMP
- The applicant should provide more details regarding the survey (i.e. timelines, outcomes for success, follow up questionnaires) to evaluate effectiveness of risk minimisation. The applicant will commit to providing full details of this post-authorisation study at a later date as a post-approval commitment. The study is anticipated to start in 2Q 2017. Category 3, RMP
- The applicant will conduct a post approval methodology study to investigate the retroviral insertion site analysis. Category 3, RMP

Recommendation: The applicant is strongly encouraged to seek collaboration with the EBMT registry and shall provide a concept protocol to the EBMT Inborn Errors Working Party or other relevant group of the Strimvelis product registry protocol approved by PRAC. The applicant will provide updates to EMA regarding progress toward creation of a patient registry with EBMT via the PBRER.

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

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	Autoimmunity Unsuccessful response to gene therapy Risks related to medical or surgical procedures (*e.g.* central venous catheter) Risks related to short shelf-life of product
Important potential risks	Malignancy due to insertional oncogenesis (*e.g.* leukaemia, myelodysplasia) Non-immunologic manifestations of ADA-SCID (*e.g.* hepatic steatosis cognitive defects, behavioural abnormalities, hearing impairment) Risks related to residuals present in the drug product administered to the patient Hypersensitivity to the product Replication competent retrovirus
Missing information	Lack of data in neonates Lack of data in adolescents Lack of immunogenicity data No reproductive toxicity studies or embryofetal development studies Lack of data in delayed onset or late onset ADA-SCID

Pharmacovigilance plan

Study/activity Type, title and category (1-3)	**Objectives**	**Safety concerns addressed**	**Status (planned, started)**	**Date for submission of interim or final reports (planned or actual)**
Adenosine Deaminase Severe Combined Immunodeficiency (ADA-SCID) Registry for Patients Treated with Strimvelis Gene Therapy: Long-Term Prospective, Non-Interventional Follow-up of Safety and Efficacy (200195) Category 1	The objective of the patient registry is to further characterise the long term safety and efficacy for GSK2696273 in ADA-SCID patients.	Autoimmunity Unsuccessful response to gene therapy Risks related to medical or surgical procedures (*e.g.* central venous catheter) Malignancy due to insertional oncogenesis (*e.g.* leukaemia, myelodysplasia) Non-immunologic manifestations of	Planned	Final report: Q4 2037*

Study/activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
		ADA-SCID (*e.g.* hepatic steatosis cognitive defects, behavioural abnormalities, hearing impairment) Risks related to residuals present in the drug product administered to the patient Hypersensitivity to the product Replication competent retrovirus Lack of data in neonates Lack of data in adolescents Lack of immunogenicity data No reproductive toxicity studies or embryofetal development studies Lack of data in delayed onset or late onset ADA-SCID		
Long-term follow up of patients from study AD1115611 Category 3	Long-term follow-up of patients treated in the GSK2696273 clinical development programme.	Autoimmunity Unsuccessful response to gene therapy Risks related to medical or surgical procedures (*e.g.* central venous catheter) Malignancy due to insertional oncogenesis (*e.g.* leukaemia, myelodysplasia) Non-immunologic	Started	Final report: Q1 2020

Study/activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
		manifestations of ADA-SCID (*e.g.* hepatic steatosis cognitive defects, behavioural abnormalities, hearing impairment) Risks related to residuals present in the drug product administered to the patient Hypersensitivity to the product Replication competent retrovirus Lack of immunogenicity data		
Effectiveness of educational materials provided to HCPs/PIDs and parents/carers. Category 3	Evaluation of the effectiveness of additional risk minimisation measures (*e.g.* educational materials) will include conducting surveys to HCPs/PIDs and parents/carers of children who are treated with GSK2696273.	Autoimmunity Unsuccessful response to gene therapy Malignancy due to insertional oncogenesis (*e.g.* leukaemia, myelodysplasia)	Planned	Final report: Q1 2021
A post-approval methodology study to investigate retroviral insertion site analysis Category 3	Evaluation of insertion site analysis to predict malignancy due to insertional oncogenesis (*e.g.* leukaemia or myelodysplasia)	Malignancy due to insertional oncogenesis (*e.g.* leukaemia, myelodysplasia)	Planned	Final report: Q4 2024

* - Timelines are contingent upon uptake of the registered product.

Risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Autoimmunity	Proposed text in SmPC: Section 4.4 Special warnings and precautions for use *"Patients can develop autoimmunity. 67% (12 of 18) of Strimvelis treated patients had either autoimmune antibodies or other manifestations (e.g. autoimmune thrombocytopenia, autoimmune aplastic anaemia, autoimmune hepatitis and Guillain-Barré syndrome) (see section 4.8)."* *Section 4.8 Undesirable Effects* *Autoimmune adverse reactions include:* *Very common: Antinuclear antibody positive and hyperthyroidism.* *Common: Antineutrophil cytoplasmic antibody positive, autoimmune aplastic anaemia, autoimmune haemolytic anaemia, autoimmune hepatitis, autoimmune thyroiditis, autoimmune thrombocytopenia, Guillain-Barré syndrome and smooth muscle antibody positive.* *"Immune reconstitution* *All the identified adverse reactions in the table (apart from those potentially related to busulfan) are considered to be related to immune reconstitution, due to their nature and timing. These autoimmune adverse reactions were reported for subjects post-gene therapy. The majority were reported during the 3 month to 3 year follow-up period and resolved, with the exception of hypothyroidism and positive ANA tests. In addition, the allergy related adverse reactions in the table were reported mostly during the 3 month to 3 year follow-up period."*	Educational materials for parents/carers and HCPs
Unsuccessful response to gene therapy	Proposed text in SmPC: Section 4.2 Posology and method of administration *"Posology* *The recommended dose range of Strimvelis is between 2 and 20 million CD34+ cells/kg.* *If the product contains less than 2 million CD34+ cells/kg, the treating*	Restricted prescription Controlled access – product consent form Educational materials for parents/carers and HCPs

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	physician should make a decision whether to proceed with administration, based on an individual benefit risk assessment. Treatment failure was observed in a patient treated in the clinical trials with <2 millions CD34+ cell/kg." Section 4.4 Special warnings and precautions for use *"Patients should be closely monitored for the occurrence of severe and opportunistic infections, immune reconstitution parameters and the need for replacement intravenous immunoglobulin (IVIG); in case of lack of response, it is recommended to introduce other ADA-SCID treatments under the supervision of a physician.* *There have been cases where treatment with Strimvelis has been unsuccessful. Some patients have had to resume long-term enzyme replacement therapy and/or receive a stem cell transplant.* *The long term effects and durability of response to Strimvelis on ADA-SCID are unknown."*	
Risks related to required medical or surgical procedures (*e.g.* central venous catheter)	Proposed text in SmPC: Section 4.4 Special warnings and precautions for use *"Adverse events related to the use of central venous catheters (CVCs) have been reported (e.g. serious CVC infections and thrombosis in the device). Patients should be closely monitored for potential catheter-related events."*	None proposed
Risks related to the short shelf-life of the product	Proposed text in SmPC: Section 4.4 Special warnings and precautions for use *"Stage two quality control results will only be available after the product has been infused. If clinically relevant quality issues, such as out of specification results, are identified after Strimvelis has been infused, the treating physician will be notified. The physician should monitor and/or treat the patient as appropriate."*	None proposed
Malignancy due to insertional oncogenesis (*e.g.*	Proposed text in SmPC: Section 4.3 Contraindications	Restricted prescription Controlled access – product

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
leukaemia, myelodysplasia)	*"Current or previous history of leukaemia or myelodysplasia."* Section 4.4 Special warnings and precautions for use *"No cases of leukaemia or myelodysplasia have been reported following treatment with Strimvelis. However, vector insertions into chromosomal regions previously associated with leukaemia in comparable trials of gene therapy in Wiskott Aldrich Syndrome, X-SCID and Chronic Granulomatous Disease have been documented. Retroviral insertion sites (RIS) have been detected adjacent to or within CCND2 and LMO2 and there is a potential risk of leukaemic transformation following treatment with Strimvelis. It is recommended that patients be monitored long term with at least annual visits for the first eleven years and then at 13 and 15 years post-treatment with Strimvelis, to include a complete blood count with differential biochemistry and thyroid stimulating hormone."*	consent form Educational materials for parents/carers and HCPs
Non-immunologic manifestations of ADA-SCID (*e.g.* hepatic steatosis, cognitive defects, behavioural abnormalities, hearing impairment)	Proposed text in SmPC: Section 4.4 Special warnings and precautions for use *"Non-immunological manifestations of ADA-SCID may not respond to Strimvelis."*	None proposed
Risks related to residuals present in the drug product administered to the patient	Proposed text in SmPC: Section 4.3 Contraindications *"Hypersensitivity to the product or to any of the excipients listed in section 6.1."* Section 4.4 Special Warnings and Precautions for Use *"Strimvelis should be used with caution in patients with hypersensitivity to aminoglycosides or bovine serum albumin."* Section 6.1 List of excipients *"Sodium chloride."*	None proposed
Hypersensitivity to the product	Proposed text in SmPC: Section 4.3 Contraindications *"Hypersensitivity to the product or to*	None proposed

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	any of the excipients listed in section 6.1." Section 4.4 Special warnings and precautions for use *"Strimvelis should be used with caution in patients with hypersensitivity to aminoglycosides or bovine serum albumin."* Section 6.1 List of excipients *"Sodium chloride."*	
Replication competent retrovirus	None	None proposed
Lack of data in neonates and adolescents	Proposed text in SmPC: Section 4.4 Special warnings and precautions for use *"Strimvelis should be used with caution in patients older than 6 years and 1 month and younger than 6 months as there are no data from clinical trials in these age ranges. Older patients are typically less able to donate high numbers of CD34+ cells which may mean that older patients cannot be treated. Successful generation of T cells after Strimvelis is also likely to be affected by residual thymic function which can become impaired in older children. Use of Strimvelis in patients older than those previously studied should be carefully considered and reserved only for occasions where all other reasonable treatment options have been exhausted."*	None proposed
Lack of immunogenicity data	Proposed text in SmPC: Section 4.4 Special warnings and precautions for use *"No immunogenicity testing has been conducted with Strimvelis."*	None proposed
No reproductive toxicity studies or embryofetal development studies	Proposed text in SmPC: Section 4.6 Fertility, pregnancy and lactation *"Women of childbearing potential* *As Strimvelis will be administered following busulfan conditioning, patients of childbearing potential must use reliable barrier contraception during administration of Strimvelis*	None proposed

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	and for at least 6 months afterwards. *<u>Pregnancy</u>* *No clinical data on exposed pregnancies are available.* *Reproductive and developmental toxicity studies were not performed.* *Strimvelis should not be used during pregnancy.* *<u>Fertility</u>* *There are no data on the effects of Strimvelis on human fertility. Effects on male and female fertility have not been evaluated in animal studies."*	
Lack of data in delayed and late onset ADA-SCID	Proposed text in SmPC: <u>Section 4.4 Special warnings and precautions for use</u> *"Strimvelis should be used with caution in patients older than 6 years and 1 month and younger than 6 months as there are no data from clinical trials in these age ranges."*	None proposed

Conclusion

The CHMP, CAT and PRAC considered that the risk management plan version 1.5 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. Significance of paediatric studies

The CAT/CHMP is of the opinion that study AD1115611, which is contained in the agreed Paediatric Investigation Plan PIP P/0190/2014 and has been completed after 26 January 2007, is considered as significant.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Labelling exemptions

A request to omit certain particulars from the labelling as per Art.63.3 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group.

Based on art. 63(3), the applicant requested the use of minimum particulars on the label that would be inserted into the pouch of the EVA bag. Even if the bag is 50mL, the company claimed there was no space for full particulars; they proposed to display the name of the medicinal product, route of administration, and batch specific information (lot number, patient ID, total cells, and CD34+ cells/kg body weight). The use of minimum particulars would also allow for the batch specific information to be clearly inserted by hand.

The particulars to be omitted as per the QRD Group decision described above will however be included in the Annexes published with the EPAR on EMA website, and translated in all languages but will appear in grey-shaded to show that they will not be included on the printed materials.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Strimvelis (autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence) is included in the additional monitoring list as it contains a new active substance and has a PASS imposed.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

Benefits

Beneficial effects

There is a compelling evidence of the efficacy of Strimvelis with respect to the primary endpoint, as a result of 100% survival (median 7 years) in both the pivotal and integrated population, since the studied condition is fatal, ADA-SCID, in the first year of life (Hassan et al 2012). This survival rate also appears to be superior to SCT from MUDs (67%) after a median follow-up of approximately 6.5 years as well as the HLA-matched sibling and family donor SCT (86% and 83%, respectively). This was also the case for intervention-free survival although the figures were somewhat lower, 92% and 82% respectively for the pivotal and integrated populations. The beneficial effects from the key secondary endpoints were consistent with the increased survival, including the decrease in the rate of serious infections compared to the baseline rate.

There was also evidence of immune reconstitution with an increase in CD3+ T cells as well as T cell subsets and some evidence of thymopoiesis as well as of peripheral T cell function, as evidenced by T cell proliferation. The numbers of CD19+ B cells was however, much variable, being lower than baseline, nevertheless, B cell function appeared to be present as evidenced by slight increases in immunoglobulin production with respect to IgM and IgA. However, levels of IgG remained unchanged. In addition, antibody response to vaccination and decrease in the requirement of IVIG, was also observed in some patients. Additionally there was an evidence of engraftment of genetically modified cells, production of the deficient ADA enzyme to clinically therapeutic levels, and the functionality of

the enzyme by the modification of the systemic metabolic defect with achieving below toxic levels of the purine metabolites, which was a key secondary efficacy endpoint.

Uncertainty in the knowledge about the beneficial effects

The absence of a comparator in the clinical trials hampers the reliable evaluation of intervention-free survival, which is more clinically meaningful especially since the pivotal study is a single-arm single centre study. In this respect, comparison with a historical control, although appropriate in the circumstances, is less informative than an active control.

Out of a total of 20 patients, 5 patients (25%) appeared to have responded unsuccessfully to gene therapy and required rescue treatment with PEG-ADA. However, based on the data available to date, it is not entirely clear which patients are likely to respond to gene therapy and this figure seems remarkably similar to that reported in the literature, as regards patients requiring restarting PEG-ADA after gene therapy. In the last stage of the procedure, the applicant updated the information, including all the Strimvelis treated patients up to March 2016, overall 6/22 (27%) received PEG-ADA as rescue medication.

The use of Strimvelis in post-marketing is expected to bring more information on this issue. Although there is a good evidence of B cell functionality, it is notable that unlike T cells, B cell reconstitution may be limited in comparison, which could impact on the requirement of IVIG after gene therapy. It is uncertain if the T lymphocyte increase could be due to other mechanisms such as expansion in central non-thymic organs (bone marrow or lymph nodes) or in the periphery of the immune system. The applicant has referred to data that would suggest that T-cell reconstitution after gene therapy, like allogeneic stem cell transplant, might be driven by both, the de novo thymopoiesis and the homeostatic expansion of naïve and memory T cells.

In addition, at this stage it is not possible to predict which patients will respond to gene therapy and further long term follow up in the registry is considered necessary before firm conclusions can be drawn. It should be noted that initial trials in gene therapy were confounded due to concomitant administration of PEG-ADA and therefore it is important to ascertain responders to gene therapy. The applicant will evaluate intervention-free survival in the registry, as requested by the CAT/CHMP.

Risks

Unfavourable effects

Compared to the SCT, the immune reconstitution appears to be much slower with gene therapy. Thus, there is a risk of development of significant infections, especially during the first year after the treatment. Notably, there could be a significant risk of autoimmunity, which needs to be managed by the treating specialist, as the majority of patients in the integrated population developed anti-neutrophil antibody-induced neutropenia, autoimmune thrombocytopenia, autoimmune aplastic anaemia, autoimmune hepatitis, Guillain-Barre syndrome and aplastic anaemia. This could potentially precipitate treatment failure as noted in 2 patients who required rescue treatment with PEG-ADA. In addition, there were multiple clinical allergy related AEs in a subgroup of patients who had increased IgE level and eosinophilia.

Furthermore, event ought the immunogenicity due to pre-existing anti-ADA antibodies that might arise from PEG-ADA treatment is considered unlikely, the effect of inhibitory antibodies with respect to treatment failure cannot be entirely excluded in some cases. Thus, the applicant agreed to monitor immunogenicity in the patients who will entered in the registry, as requested by the CAT/CHMP.

Although SAEs were noted in the majority of subjects, none of the SAEs were judged to be related to treatment. However, it is likely that neurologic, CNS and hearing events may not fully respond to gene therapy, as it has also been noted for the ERT and SCT. Upon Strimvelis administration, there is also an increase in hepatobiliary events, especially cases of hepatic steatosis which were detected during a long term follow up. A few patients developed AEs with respect to decreased T-cell V beta repertoire, pulmonary mass, lipofibroma, electrophoresis protein abnormal, as well as skin papilloma. All SAEs have been included in the SmPC in order to inform the physicians about the possible side effects. Moreover, several post-authorisation measures have been agreed in order to monitor amongst others the events of hepatotoxicity and immunotoxicity.

The applicant also acknowledged that there is limited experience about recommending the pre-treatment conditioning with busulfan. In the frame of the agreed long-term follow up post-authorisation registry, the applicant has committed to conduct an exploratory analysis in order to investigate the relationship between busulfan AUC, captured at baseline, and clinical outcome (e.g. survival, intervention free survival and lymphocyte count differentials).

Uncertainty in the knowledge about the unfavourable effects

With the use of retroviral vectors, there is a concern of a potential for insertional mutagenesis and oncogenesis. However, as yet, no cases of leukaemia, myelodysplastic syndrome or malignancy have developed after the treatment with Strimvelis, which is cautiously reassuring from a safety perspective. Nevertheless, as ADA is an enzyme, and is not involved in processes controlling cell proliferation/apoptosis, tight gene expression regulation has not been shown to be crucial yet, and overexpression is unlikely to cause significant side effects. Nevertheless, it is important to consider long term follow-up with respect to the evaluation of the risk of oncogenesis and hence, the CAT/CHMP requested the applicant to conduct a post approval methodology study to investigate the retroviral insertion site analysis. In addition, estimates of clonal abundance and comprehensive identification of integration site locations using current methods are compromised by several types of recovery biases and sources of variability. Therefore, retroviral insertion site is currently unsuitable as an individual subject monitoring safety tool. Retroviral insertion site analysis has been studied at a single point in time, nevertheless, to test the persistence and contribution of identical insertion sites in a given cell lineage, patients need to be studied at different time points after gene therapy. Therefore, an assay with non-restrictive platforms will be developed by the applicant to address this issue.

Furthermore, it cannot be assumed that adverse events will not occur in the long term and accordingly, patient monitoring needs to be continued to ensure the long term safety of gene therapy with Strimvelis. The CAT/CHMP recommended that all patients are enrolled in a registry for monitoring for at least 15 years from the time of Strimvelis administration. This long-term follow up via a specific registry is considered important to benefit/risk of Strimvelis.

Effects table

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Survival	Survival with respect to death from any cause at 3 years		Strimvelis	None	100% survival Comparison with historical control	Discussion on clinical efficacy
Infections	Rate of infection estimated as number of infections over person-years of observation (free from infection) after the first 3 months after treatment administration (after gene therapy) or before gene therapy	Change in rate of severe infection	Strimvelis	None	1.1 vs 0.429 (P=0.005)	Discussion on clinical efficacy
Change in T-lymphocyte count	Comparison of CD3+ T lymphocytes between baseline and Year 3	CD3+ T lymphocytes GM ratio nmol/m	Strimvelis	None	3 vs 6,5 (0.38) CI 3.08-13.64 <0.001	Discussion on efficacy
Metabolic detoxify-cation Y or N	Adequate 'systemic' metabolic detoxification was classified as levels of purine metabolites (dAXP=dAMP+dADP+dATP) in RBC <100 nmol/mL. at baseline and year 3		Strimvelis	None	O vs 11/11(100%) CI 72-100% <0.001	Pharmacodynamics section

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Unfavourable Effects						
	Autoimmunity				12/18 (66%)	Discussion on safety
	Hepatobiliary events				13/18 (72%)	Discussion on clinical safety

Benefit-risk balance

Importance of favourable and unfavourable effects

ADA-SCID is primarily diagnosed in very young paediatric patients who, without treatment, rarely survive beyond 1-2 years. Patients enrolled in the pivotal study lacked a healthy HLA-identical sibling donor and some of them failed after at least 6 months of treatment with PEG-ADA due to intolerance, allergic reaction or autoimmunity or because the ERT was not a lifelong therapeutic option. In these patients, Strimvelis has shown to reach a 100% survival rate that was maintained after a median patient follow-up period of 7 years. These data are comparable to the ones obtained with HLA-identical family donor. The figures of intervention–free survival, though lower, are also comparatively superior with gene therapy. In addition, there are other factors which are also bypassed with gene therapy with respect to the use of full myeloablation as well as GvHD after allogeneic SCT. The reduction in serious infection rate as well as immune reconstitution together with detoxification of metabolites is also significant as these are common causes of mortality in this population of patients. With respect to unfavourable effects, the occurrence of autoimmunity can significantly impact on outcome of treatment with gene therapy and may require the administration of rescue treatment. Additionally, the risk, albeit comparatively smaller, with respect to insertional mutagenesis, may give rise to leukaemia, MDS and malignancy which may impact on survival.

Benefit-risk balance

The totality of data from the clinical programme of Strimvelis demonstrate compelling evidence for efficacy since patients with early and delayed onset ADA-SCID rarely survive beyond 1 to 2 years unless immune function is restored either with SCT or PEG-ADA. With respect to safety, the risk of insertional mutagenesis would appear to be comparatively smaller than has been observed in other diseases with similar vectors possibly because ADA is an enzyme, and is not involved in processes controlling cell proliferation/apoptosis, therefore tight gene expression regulation has not yet been shown to be crucial, and overexpression is unlikely to cause significant side effects. While autoimmunity is an issue, and it will be evaluated in the registry, it would appear to be manageable with additional standard therapy. Accordingly, from a clinical perspective, the benefit/risk balance of Strimvelis is considered positive.

Discussion on the benefit-risk balance

Strimvelis has been shown to increase life survival up to 100 % in a disease, ADA SCID, with which patients rarely survive beyond 1-2 years. This has shown to be possible with provision of evidence of engraftment of gene modified cells in the bone marrow as a result of which, the deficient ADA enzyme is produced to levels which are considered clinically therapeutic. In addition, evidence that the enzyme is functional is determined by the detoxification of metabolites to clinically relevant levels. It is fully recognized that immunological reconstitution alleviates the imminent problem and provides a curative opportunity to this life-threatening situation. The consequences of the administration of Strimvelis on

immune reconstitution have resulted to be effective and are the main contribution to the clinical benefit. Thus, the basis for the increased survival would appear to be robust, while the key secondary endpoints are fully supportive and consistent with the increased survival. A decrease in the rate of severe infections is consistent with the immune reconstitution of mainly T cells and to a much lesser extent B cells whose functionality support the beneficial effects outlined above.

Strimvelis was shown to be relatively well tolerated although data are limited due to the small number of patients studied. There is potential risk of insertional mutagenesis and oncogenesis with this type of retroviral vector. Nevertheless, as yet, no cases of leukaemia, myelodysplastic syndrome or malignancy have developed which is cautiously reassuring from a safety perspective and post-authorisation measures are in place to monitor such events. However, as ADA is an enzyme, and is not involved in processes controlling cell proliferation/apoptosis, tight gene expression regulation has not to date been shown to be crucial and overexpression is unlikely to cause significant side effects. With respect to the evaluation of the potential risk of oncogenesis, a long term follow-up with via the registry and a separate study was agreed. This is considered key to the positive benefit/risk of Strimvelis. Additionally, autoimmunity, may impact on the outcome of gene therapy due to immune reconstitution which may require rescue treatment. These potential risks will be followed up and monitored by the applicant who committed to a number of post-authorisation measures, as requested by the CHMP.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described above.

4. Recommendations

Outcome

Based on the draft CHMP opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Strimvelis in the treatment of patients with severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID), for whom no suitable human leukocyte antigen (HLA)-matched related stem cell donor is available, is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the

agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Prior to launch of Strimvelis in each Member State, the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational materials for parents/carers and health professionals, restricted prescription details and controlled access/product consent form, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

Strimvelis will be administered at a specialist transplant centre, and by physicians with previous experience in the treatment and management of patients with ADA-SCID and the use of autologous CD34+ *ex vivo* gene therapy products. A completed product consent form is required prior to initiating treatment.

- **Obligation to complete post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measure:

Description	**Due date**
Non-interventional PASS: In order to investigate the long term safety and efficacy of Strimvelis gene therapy, the MAH should conduct and submit the results of a long term prospective, non-interventional follow up study using data from a registry of patients with adenosine deaminase severe combined immunodeficiency (ADA-SCID) treated with Strimvelis. The MAH will follow up on the risk of immunogenicity, insertional mutagenesis and oncogenesis as well as hepatic toxicity. The MAH will review the occurrence of angioedema, anaphylactic reactions, systemic allergic events and severe cutaneous adverse reactions during the FU period, particularly in those patients who had unsuccessful response and received ERT or SCT. The MAH will also evaluate intervention-free survival.	The MAH shall plan to include regular progress reports of the registry in the PSUR and provide interim study reports every 2 years until the registry finishes. Interim registry reports shall be submitted every 2 years. The final clinical study report should be submitted after the 50th patient has 15 year follow-up visit; Q4 2037.

The CHMP endorse the CAT conclusion on the obligation to complete post-authorisation measures as

described above.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the CAT review of data on the quality properties of the active substance, the CAT considers that Autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence is qualified as a new active substance.

The CHMP endorse the CAT conclusion on the new active substance status claim.

Paediatric Data

Furthermore, the CAT/CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan PIP P/0190/2014 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

In accordance with Article 45(3) of Regulation (EC) No 1901/2006, significant studies in the agreed paediatric investigation plan PIP P/0190/2014 have been completed after the entry into force of that Regulation.